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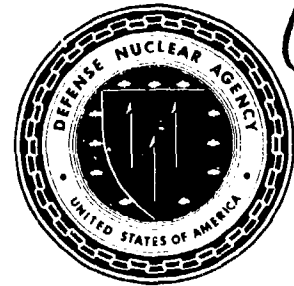
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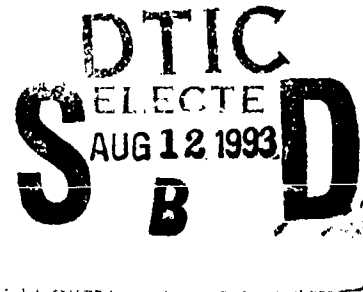
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Technical Ramifications of Inclusion of Toxins in the Chemical Weapons Convention (CWC), Supplement

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Fort Detrick, MD 21702-5011**

August 1993



Technical Report

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EXECUTIVE SUMMARY

Endogenous bioregulators, or bio-mediators, are naturally-occurring, highly potent chemical compounds that control biological processes. Many of them are small peptides or lipid derived substances that are relatively easy to synthesize and manipulate chemically. In this report, the bioregulators are discussed in terms of their importance in affecting biological activity by exogenous administration and the technical ramifications of including them in international treaties that control their offensive applications. Mediators that have limited potential for incapacitation or principally affect biological functions that are not of concern of such treaties are included only for clarity.

Regulators can act on the same cell that produces them, and/or on neighboring cells, and/or on distant sites. The actual effect of any mediator may be the result of the interaction of peripheral and central effects of the mediator on one or more class of specific receptors as well as the interaction of one mediator with other mediators that may modulate the response. These interactions make an a priori prediction of a specific action of a new or modified mediator most difficult. Endogenous mediators do not induce irreversible changes and are rapidly cleared from the circulation. Most are cleared during one pass of the blood through the lungs by clearance mechanisms in the lungs. Thus, the rapid clearance and metabolism of mediators limit their biological activity.

Aerosol delivered neuropeptide mediators must transit from the lungs to the circulation and then breach the blood-brain barrier to induce an effect on the central nervous system; to date, this whole sequence of events has not been accomplished and is not foreseen in the near future. Endogenous mediators that, when delivered by aerosol, need only reach the circulation to induce a cardiovascular effect or those mediators that can induce a direct effect on the respiratory system exist today. The neuropeptides have traditionally received the greatest attention by those concerned with the potential threat posed by the endogenous mediators. The lipid mediators have been largely ignored, but they produce a biological effect (asthma like attack) when administered by aerosol at lower dosages than the neuropeptides, are as readily (or more so) synthesized as the neuropeptides, and have many other characteristics that should arouse concern for their inappropriate application.

All of the peptide mediators can be and are being synthesized, as are many of the lipid-based mediators. Most of these are available, commercially, for both human and veterinary use. Most, if not all, of the peptide mediators may be obtained by recombinant DNA (rDNA) technology. Almost all natural lipid mediators, including a large number of analogs, have now been

chemically synthesized. Modifications are possible and a plethora of synthetic analogs have been designed. This has resulted in compounds with desirable properties of solubility, increased stability against metabolic degradation, and selective biological activity. Extended shelf-life needs for animal and human pharmaceutical applications of these products have driven the research for storage stability. Stability under conditions associated with offensive use is unknown. While there is no scientific verification of the development of metabolically stabilized mediators that can have severe incapacitating or lethal effects when delivered by aerosol, this is theoretically within scientific capability of today's technology.

Because of the importance of the bio-mediators in medicine, standardized assays and methodologies are available. The difficulty that could be encountered would be in recognizing and assaying "aberrant or unusual" mediators, in quantitating excessive levels of mediators in the body, or detecting malicious delivery of the mediators. No assay methodologies have been reported for the detection of biomediators in air.

Because the mediators are part of the natural physiologic functioning of animals and man, the mediators are used extensively in veterinary and human medicine. Indeed, a whole line of the pharmaceutical industry has developed around the production of peptide and lipid mediators for animal and human use. Much of the research on novel compounds is being driven by this industry.

The endogenous mediators do not meet the criteria for inclusion under Schedule 1 or 2 Chemicals in the CWC text: they were not previously weaponized; they are not precursors of such agents; they do not have high potential for use as weapons due to toxicity; and they all have widespread legitimate use in research and medicine. Inclusion in Schedule 3 would have no impact on the legitimate use of these compounds. But, the impact on peaceful use of these substances is not the issue that concerns many people. Rather, the concern deals with the detection and monitoring of illicit use of these substances.

In summary, the bioregulators have widespread use in medicine and are readily available from commercial sources in reasonable quantities. Most of the mediators can be chemically synthesized and/or produced by rDNA technology. Standardized assay procedures are available or published for the known and characterized mediators, but not for the myriad synthetic analogs that are or potentially are available. Procedures are neither established to differentiate physiologic levels from pathological levels, nor are methodologies available for detection of the mediators in air and, like toxins, the mediators leave no residuum or signatures.

Conversion Table

Multiply _____ by _____ To Get
To Get _____ by _____ Divide

dalton (Da)	1.000 000 x E -3	kilo dalton (kDa)
gram (kg)	1.000 000 x E +15	femtogram (fg)
inch	2.540 000 x E -2	meter (m)
kilogram (kg)	1.000 000 x E +6	milligram (mg)
kilogram (kg)	1.000 000 x E +3	gram (g)
kilogram (kg)	1.000 000 x E +9	microgram (μg)
kilogram (kg)	1.000 000 x E +12	nanogram (ng)
kilogram (kg)	1.000 000 x E +15	picogram (pg)
kilogram (kg)	1.000 000 x E -3	metric ton (mt)
liter (l)	1.000 000 x E +6	microliter (μl)
liter (l)	1.000 000 x E +3	cubic centimeter (cc)
liter (l)	1.000 000 x E +3	milliliter (ml)
nanometer (nm)	1.000 000 x E -9	meter (m)
meter (m)	1.000 000 x E +2	centimeter (cm)
micrometer (μm)	1.000 000 x E -6	meter (m)
mole (M)	1.000 000 x E +12	pico mole (pM)
mole (M)	1.000 000 x E +6	micro mole (μM)
mole (M)	1.000 000 x E +18	attomole
mole (M)	1.000 000 x E +15	femtomole
mole (M)	1.000 000 x E +9	nano mole (nM)
ounce	2.834 952 x E -2	kilogram (kg)
quart (US liquid)	9.463 333 x E -1	liter

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SECTION 1

INTRODUCTION

Bioregulators are naturally-occurring chemical substances, usually peptides, involved in the regulation of metabolic, physiologic and neural activities (166,167,168,186,187,188,189,190). Bioregulators have also been referred to as endogenous mediators and as "agents of biological origin" or ABOs (Spertzel, personal communication). In some reports, bioregulators have been used in a more restricted sense to mean only the neuropeptides (165). In this report, bioregulators and endogenous mediators will be used as synonymous terms to mean endogenous substances or their analogs that regulate, mediate or modulate biological activity at the cellular level.

Many highly potent chemical compounds that control biological processes have been identified (166,167,168,171,172,175,177,180,181,182,183,184,185,186,187,188,189,190,191,192). Many of these are small peptides and lipid derived substances that are relatively easy to synthesize and manipulate chemically (165,167,175,176,180,188,189,190,191). It is now feasible to produce sizeable quantities (kg to hundreds of kg) of these compounds (165,176). Thus, it is important to realize that bioregulators could be used as warfare agents, especially if physical and/or mental incapacitation, rather than lethality, were among the sought-after effects (165).

The majority of bioregulators are peptides (168,171,172,173,175,180,181,182,183,184,186,187,192,177). In spite of their different functions, all peptides are basically polymers of different amino acids (165). By analogy, peptides are like beads on a string. There are over twenty possible choices for each position. Peptides differ from many other polymers in having a strictly defined length and sequence of amino acid monomers. These are acquired from their unique mode of biosynthesis. They differ from each other in that each peptide has a unique amino acid sequence. A new peptide is made every time a single amino acid is changed. These changes can alter the biological activity of the peptide (165) and the potential for use as a warfare agent.

In this report, the endogenous mediators are discussed in terms of their importance in affecting biological activity by exogenous administration and the technical ramifications of including them in international. Those mediators, that have limited potential for incapacitation or principally affect functions that are not of concern of such treaties, are included only for clarity, where appropriate, and are not discussed, e.g., prolactin and oxytocin and their effects on lactation.

SECTION 2

BIOREGULATORS

Regulators can act in an autocrine manner (on the same cell that produces them), and/or paracrine manner (on neighbor cells), and/or endocrine manner (on distant sites) (187,192). The actual effect of any mediator may be the result of the interaction of peripheral and central effects of the mediator on one or more class of specific receptors for that mediator as well as the interaction of one mediator with another mediator that may modulate the response (192). These interactions make an a priori prediction of the effects of a new or modified mediator most difficult.

2.1 GENERAL CONSIDERATIONS.

In experimental studies, endogenous mediators are generally administered subcutaneously (sc), or locally in the circulation to observe local and systemic effects, or intracerebro-ventricularly (icv) to observe central and centrally mediated systemic effects; some, especially in the study of pulmonary effects, have been administered as aerosols by inhalation (182,183,184). Some, with therapeutic applications, have been investigated for administration by ingestion (176).

The route of administration is not of trivial significance. Not only does the dose required to obtain a specific effect vary with the route of administration but also the administration by different routes often results in different effects. For example, intravenous administration of endothelin-1 elicits a dose-dependent bronchoconstriction which is accompanied by a rapid and marked rise in mean-arterial blood pressure, whereas by aerosol, the peptide appears to act on airway smooth muscle cells directly without causing significant changes in blood pressure (44,51,73).

Ingestion has been an effective route for some peptide mediators that are effective systemically, e.g., insulin, and for some lipid mediators, e.g., some prostaglandins (180). Central effects from ingested or any other peripherally administered mediators, if they occur, require more than 1000 times the icv dose for any measurable effect (180, 188,189,190,191). For example, 2500 times more dermorphin and dermorphin analogues are required sc than icv to produce a particular effect and approximately 100 times the sc dose if administered orally (23,45,66,76,129,132). Where central effects are observed from parenterally administered mediators, they are generally believed to be due to secondary interactive effects resulting from stimulation of other mediators (180,188,189,190, 191). Some smaller mediators may cross the blood-brain barrier by special transport mechanisms, albeit not efficiently (192).

Endogenous mediators delivered by aerosol would have to transit from the lungs to the circulation and then breach the blood-brain barrier to induce a central effect (185); to date, this entire sequence of events has not been accomplished and is not foreseen in the near future. The endogenous mediators that, when delivered by aerosol, need only reach the circulation to induce a cardiovascular effect or those mediators that can induce a direct effect on the respiratory system, exist today (167,188, 189,190,191). Their limitation on biological activity is their rapid clearance from the circulation, much of which occurs in one pass of the blood through the lungs by clearance mechanisms in the lungs.

2.1.1 Air-lung-blood Barrier.

In the alveoli-capillary region of the lung, air and blood are separated by a barrier composed of alveolar epithelial and capillary endothelial cells, and of some interstitial elements (185). Diffusible substances to be exchanged between air and blood have to pass this tissue space; they enter and leave the tissue across its external (air) and internal (blood) boundaries or interfaces.

Substances, delivered to the lungs as gasses, vapors, aerosols, or dusts must either be capable of direct effect on the lungs or transit this barrier to produce an effect by action elsewhere in the body (185). Diffusible gases and vapors, if they escape entrapment, filtration and clearance by elements in the upper respiratory tract and tracheobronchial tree, can transit this barrier. Particulate matter in dusts and aerosols, in addition to entrapment, filtration and clearance by elements in the upper respiratory tract and tracheobronchial tree, must either be actively transported across this barrier, penetrate through relatively few holes that inevitably exist in this barrier at any one time or create damage to this barrier and thus reach the blood.

The lung parenchyma is rich in various metabolic enzymes that function to breakdown substances that are produced naturally in response to foreign stimuli (endogenous mediators) or that have escaped the clearance mechanisms of higher portions of the respiratory tract (185). Thus, relatively few substances that act elsewhere in the body are successful in transiting this barrier at the concentrations required to be effective. Those substances that must act centrally then have the additional obstacle of the blood-brain barrier, see 2.1.2 below.

2.1.2 Blood-brain Barrier.

The blood-brain barrier is the term used to describe the lack of ready penetration of certain blood-borne molecules through the endothelia of brain capillaries into the brain

interstitium (173,187,192). The functional blood-brain barrier is not an absolute, impenetrable barrier to movement of molecules but rather represents a reduction in the rate at which exchange occurs between fluid compartments (187,192).

2.1.3 Receptors.

The major factor determining the response of a tissue to a mediator is the presence of a cellular receptor for the mediator and the post-receptor machinery to which that mediator receptor is coupled (166,167,168,169,171,172,173,177,185,186,188,189,190,191,192). For the peptide and lipid-derived mediators discussed in this report, the receptors are present on the plasma membrane of the cell. Receptor sites are being identified and characterized everyday. This can assist in several areas; medical applications of the bioregulators; development of novel pharmacotherapeutic compounds; and at the same time provide improved diagnostic and detector systems which would assist in identification of such bioregulators should they be used for warfare purposes.

Biologically active peptides, including the neuropeptides, and other biologically active compounds exhibit a high degree of diversity within the same group of compounds not only through the regulation of mediator production but also through mediator-receptor interaction (169,172,192). For example, 5 different subtypes of opiate receptors, δ , ϵ , κ , μ , σ , have been characterized (172). They differ in pharmacologic properties, distribution in the brain and elsewhere, and affinity for various opioid peptides (172).

2.2 CENTRAL NEUROREGULATORY MEDIATORS.

The central action of mediators are reasonably well documented, although new mediators, peptides and non-peptides, and new receptors are being reported every year (177,182,183,184,187). To have a central effect, the mediator must either be synthesized and secreted in the CNS or breach the blood-brain barrier (187). The natural occurrence of the latter is undocumented. Experimentally, such central effects of exogenous sources of neuromediators are elicited by direct injection into the CNS.

A vast number of diverse molecules (chemical messengers) are used by cells to communicate with other cells (172,173,186,187). Peptides play an important role as modulators of cellular activity in both the endocrine and nervous systems (173,182,183,184,192). The brain synthesizes and secretes peptides as neurohormonal messengers (172,187). Many peptides are present in both gut and brain tissue and are produced by many cell types throughout the body (172,182,183,184,186,187). Thus, peptides are best thought of as part of the large repertoire of possible

chemical mediators for cell-to-cell communication (186,187). Neuroregulators produced in the brain may act as neurotransmitters or co-transmitters much like acetylcholine, as neuromodulators (potentiate the effect of another transmitter) or as neuromediators (neuroregulators) affecting a specific function(s) elsewhere within the brain or peripherally via action on distal nerve fibers and receptors (172,187). In addition, neuropeptides that are secreted into the general circulation may have a direct effect via receptors (see 2.3) located on cells of other organ systems, e.g., smooth muscles of the respiratory tract (185).

2.3 SYSTEMIC ACTING MEDIATORS.

While much attention has historically been given to the neuropeptides/neuromodulators, the difficulty in penetration of the blood-brain barrier by systemically delivered mediators, remains a major hindrance to attaining central effects of systemically delivered mediators (176,192). Of potentially much greater concern are the mediators that act locally or systemically after local/systemic administration and that might impose severe debilitating/incapacitating effects when delivered in quantities much higher than physiological levels. For example, endothelin-1, delivered by aerosol, potentially could cause severe bronchospasm resulting in a life-threatening asthma-like attack (51).

Endogenous agents, including histamine, bradykinin, serotonin and some prostaglandins stimulate afferent nerve endings (c-fiber endings) as does capsaicin (toxic substance from peppers) (187). In some pathological states, many of these substances are released; thereby a central role for neuropeptides may develop in such states as asthma, pulmonary congestion (as occurs with an elevation of pulmonary vascular pressure), pulmonary embolism, and acute lung injury from various causes (187). Other effects may be identified and become known as the knowledge base of this rapidly expanding field becomes available thus contributing to the potential for their illegal use in warfare.

2.3.1 Bronchoconstriction.

Locally produced and locally inactivated mediators regulate flow through the different regions of the pulmonary circulation (187). For instance, the vasoconstriction and edemagenic actions of one group of arachidonic acid metabolites released in small amounts by the local endothelium are normally nicely balanced by the vasodilator and membrane stabilizing properties of another group. Many of the mediators have shown direct constrictor (and dilator) activity on the smooth muscle of the trachea and bronchi (187).

2.3.2 Cardiovascular Effects.

Systemic regulation is brought about by locally produced and locally active substances, by circulating substances, and by the vasomotor nerves (173,187). Substances in the circulation that bring about vasodilation include kinins and ANP (173). Circulating vasoconstrictors include vasopressin, norepinephrine, epinephrine and angiotensin II (173). Locally produced, locally acting vasoactive substances include the endothelins, lipid mediators, and endothelium-derived relaxing factor (173).

2.3.3 Gastrointestinal Effects.

Certain gastrointestinal hormones (e.g., VIP and substance P) may affect intestinal blood flow by acting as neurotransmitters or as modulators of neurotransmission rather than through direct actions on blood vessels (183). The opiate receptor agonists (endorphins, met-enkephalin, and leu-enkephalin) may also increase gastrointestinal blood flow, O_2 consumption, and motility by acting as neurotransmitters (192). Angiotensin II causes vasoconstriction and a decrease in blood flow; adrenal catecholamines have the same action (187). During severe dehydration, Arg-vasopressin (AVP) may reach high enough levels in the bloodstream to cause significant intestinal vasoconstriction (187).

2.4 ENDOGENOUS MEDIATORS: STRUCTURE AND TOXICITY.

Living cells produce a wide variety of chemical messengers that are used to communicate with other cells (187). These endogenous lipid and peptide mediators include: the cytokines (TNF, interleukins, interferons) (168,171,177); classical hormones (insulin, ACTH, etc) (182,183,184); eicosanoids (prostaglandins, thromboxanes, leukotrienes, etc.) (190,191); PAF (167); and a number of smaller peptide regulators that act on the CNS, cardiovascular, pulmonary, and other systems (180,182,183, 184). Appendix A has a more complete description of mediators, structures and functions.

2.4.1 Lipid Mediators.

Oxygenated derivatives of arachidonic acid (eicosanoids) and platelet-activating factor are lipid mediators produced by cells involved in the mediation of many inflammatory reactions including the mediation of pulmonary inflammation (167,185,190, 191). Each of the lipid mediators (prostaglandins, thromboxane, leukotrienes and PAF) interact with distinct receptors present on cell surface membranes to induce their biologic cellular response (see 2.3). The eicosanoids include the prostaglandins, the thromboxanes, the prostacyclins, and the leukotrienes. Eicosanoids display a number of biological actions with direct

implication to the microvascular bed, smooth muscle, and the process of inflammation (190,191). PAF is a potent phospholipid, autacoid mediator implicated in a diverse range of human pathologies including shock, ischemia, cardiac and systemic anaphylaxis, CNS and renal disorders, asthma, and a variety of inflammatory conditions (167). PAF-induced bronchoconstriction is mediated largely by release of sulfidoleukotrienes such as LTD4 in humans (58). A range of molecular species of PAF has been reported but it is not known if these act upon the same or different receptors. Toxicity of selected lipid mediators are shown in Table 1. Most of the lipid mediators

Table 1 - Characteristics of lipid mediators.

Mediator	Biostability	Toxicity (Total human dose)	
		Lethal	Bronchoconstriction
Prostaglandins			
PGC2		Unknown	~10 μ g
PGD2		Unknown	~10 μ g
PGE2		Unknown	~40 μ g
PGI2	2 min	Unknown	Dilator
Leukotrienes			
LTC4	<1 min	Unknown	0.6-8 μ g
LTD4	<1 min	Unknown	0.8-12 μ g
LTE4	2-4 min	Unknown	45 μ g
Thromboxanes			
TXA2	5-10 sec	Unknown	Unknown
PAF	<1 min	Unknown	45-80 μ g

induce direct effects on the respiratory system, counteracting or augmenting the action of other mediators (185,190,191). Data is lacking on lethality and/or high overdosing of these mediators by any route of administration. Induction of bronchoconstriction and other asthmatic-like symptoms in humans by several of these mediators have been reported, see Table 1.

2.4.2 Neuropeptides.

In the past few years, a growing number of neuropeptides have been identified in the CNS (182,183,184,187). Neuropeptides are present in all major brain areas (182). Many are also present in the peripheral nervous system and non-neuronal tissues like the pituitary gland, adrenal gland, gastrointestinal mucosal cells, and the pancreas (182). Several neuropeptides were originally characterized in non-neuronal cells and have been reported to be present in the CNS (182).

For convenience, the neuropeptides, in this report, have been arbitrarily classified into five groups: 1) The hypothalamic Neuropeptides are present in highest concentration in the

hypothalamus and are involved in regulating pituitary function (releasing or release inhibiting hormones) or in acting as neurohormones on the periphery (posterior pituitary hormones). They are also widely distributed in other parts of the brain and may act as neurotransmitters at extrahypothalamic sites (182). These neuropeptides include, among others: somatostatin, vasopressin, and oxytocin. 2) The pituitary neuropeptides that are characteristically synthesized by anterior pituitary cells, but several neuronal cell groups in the brain are also able to synthesize them (182). Their normal concentrations in the brain are one or two orders of magnitude lower than in the pituitary (182). 3) Three families of opioid peptides in the brain, endorphins, enkephalins, and dynorphins, are derivatives of three large precursor molecules: pro-opiomelanocortin, pre-proenkephalin, and pre-dynorphin/neo-endorphin, respectively (187). Opioid peptides are widely, but rather individually, distributed in the CNS and in the GI tract. Opioid receptors are present in the brain, GI tract and lungs. Opioid peptides have been isolated and identified from non-mammalian sources as well, e.g., dermorphin and deltorphin from frogs. 4) The brain-borne gastrointestinal hormones are peptides that are distributed in the gastric and intestinal mucosal cells and pancreatic cells. Many of them occur in the nervous system of lower species only, while others exist as native and bioactive neuropeptides in mammalian brains. These neuropeptides include, among others, substance P, neuropeptide Y, neurotensin, secretin, and insulin. 5) Other neuropeptides that are also widely distributed in the CNS, some of them in high concentrations and that also occur in non-neuronal tissues. Many neuropeptides in this group are known as strong, vasoactive substances. These neuropeptides include angiotensin II, bradykinin, and delta sleep-inducing peptide among others, see Table 2. No aerosol data is available on these peptides. Intravenous and sc data (where available) suggests that these peptides require relatively high doses (milligram quantities) except when administered directly (icv) into the CNS.

It is hard to assess the actual dose equivalency between blood-borne angiotensin and angiotensin injected into the brain (182). In order for angiotensin to get into the brain it must cross the blood brain barrier (172,187). Careful studies of Ang II in cerebrospinal fluid of rats and dogs showed that none crossed the BBB from plasma (187). Normally, the BBB would exclude large molecules from brain tissue. The physiology of the BBB produces a compartmentalization between the ventricular system and the vascular system (187). The result is that peptides in the CSF may have different effects from their effects via the blood. Bradykinin, when injected into the blood, causes vasodilation, and when injected into the brain causes vasoconstriction (31a,187).

Table 2 - Characteristics of neuropeptide mediators.

Mediator	Structure	Toxicity (total dose)			
		Subject	Route	Lethal	Br. Const.
Somatostatin	14 aa				No
Vasopressin	9 aa				?
Endorphins	31 aa		iv	>2.5 mg	No
Enkephalins	5 aa		sc	10.0 mg	No
Dynorphin A	17 aa				No
Dynorphin B	13 aa				No
Dermorphin	7 aa		sc	>5.0 mg	No
Substance P	11 aa	GP			?
Substance K	10 aa	GP			?
Neuropeptide Y	36 aa				No
Neurotensin	13 aa				No
Angiotensin II	8 aa	GP			No
DSP					No
bradykinin	9 aa	Pig			1 mg/kg

2.4.3 Endothelins.

Endothelin (ET) refers to a family of acidic, 21-amino acid peptides found in at least four distinct isoforms: ET-1, ET-2, ET-3, and endothelin β (also called vasoactive intestinal contractor) (119,138,181). ET isopeptides share sequence homology and a common structural design (138); all possess two intrachain disulfide bridges between the cysteine residues 1 and 15, and 3 and 11 (34). Endothelin-2 shows the greatest homology with ET-1 and differs at only 2 positions (119). ET-3 has 6 substitutions relative to ET-1, 4 within the smaller intramolecular loop and two substitutions adjacent to bridge forming cysteinyl residues (119). Both in structure and bioactivity, ET peptides are closely related to sarafotoxins S6 (a, b, c and d), peptide toxins isolated from the venom of an Egyptian asp (138). In vivo studies in animals have shown that inhalation of ET-1 aerosol induces a potent bronchoconstriction without significant changes in blood pressure at doses 10 to 100 times less than the lethal (15 μ g/kg) dose (112). ET-1 is more effective than ET-3 or VIC (44,79).

2.4.4 CYTOKINES.

Cytokines are polypeptides synthesized by many cells which act on a variety of tissues by changing gene expression and cellular metabolism and help sustain, amplify and regulate the cellular immune and inflammatory response to local infection (171,177). Cytokines are capable of affecting the function of virtually every cell, tissue, and organ system. They have endocrine, paracrine, and autocrine roles in the inflammatory

response and mediate changes that resemble aspects of sepsis and injury (171). Many cytokines have similar, or at least overlapping, activities. But even molecules that have a multiplicity of activities in common may have different consequences to the host (171). The pro-inflammatory cytokines are a particular group of cytokines with molecular weights between 8,000 - 25,000 Da which appear to be synthesized primarily in association with disease states or during host perturbation (171). These polypeptides are very potent molecules which, at picomolar or even femtomolar concentrations, trigger a variety of responses in cells. Cytokines appear to fall into two main groups: cytokines that act primarily as growth factors for a variety of cells and cytokines that possess pro-inflammatory properties; TNF and IL-1 are two of the most thoroughly studied pro-inflammatory cytokines (171). In vitro studies with TNF α suggest that this cytokine is a pulmonary vascular dilator and may lead to the late increases in vascular permeability seen in adult respiratory distress syndrome (177).

SECTION 3

TECHNICAL CONSIDERATIONS

The known regulatory peptides number in the hundreds, and additional peptide hormones will be found in the isolation of substances responsible for specific biologic activities or by the decoding of gene sequences (182,183,184,192). The potential number of unique amino acid sequences that are possible is immense (192). For example, if all possible combinations of the 20 amino acids were utilized, 2×10^{11} different peptides, each of 10 amino acids, could exist. A typical mammalian cell expresses genes encoding between 5,000 and 10,000 different proteins, and among some specialized cells the total repertoire is probably somewhere around 50,000 proteins (192).

For most mediators, the interaction between the mediator and the receptor is rapid and reversible, thus serving the homeostatic, physiologic need for rapid initiation and rapid termination of the mediator action (192). On any cell there are a finite number of receptors for each mediator to which the cell responds, the number varying from fewer than 100 to more than 1 million per cell (192). Mediators generally bind to their receptors with high affinity and specificity (low affinity, cross binding among related mediators does occur) (192).

Although peptides are generally of biological origin, it is possible to synthesize them chemically so that they have biological activity (165). The amino acid sequence of a given biologically active peptide usually differs slightly from species to species and sometimes within a single individual. Peptide regulators derived from animals are usually active in humans. Some bioregulators do not require the presence of all amino acids of the parent molecule to maintain biological activity. For example, the adrenocorticotropin hormone (ACTH) is a single chain peptide containing 39 amino acids. The first 24 amino acids are responsible for its biological activity. When the last 15 amino acids are removed, it does not affect its biological activity.

Mediators in general have very short life spans in the circulation; depending to some extent, on the various (and sometimes multiple) organ systems that actively degrade the mediator (182,192). Mediators in the blood that have general regulatory functions throughout the body are secreted, taken up or inactivated throughout the pulmonary circulation (and in some cases by the liver and kidneys as well) (185). For example, the half-life of bradykinin is 17 seconds, Angiotensin II is 2 - 4 minutes and ET-1 is 1.5 - 7 minutes. (ET-1 clearance is biphasic, however, with accumulation in certain tissues, mainly the lungs and kidneys, with a half-life of this second phase of around 45 minutes.) The pulmonary endothelial cells have

specific receptors and enzyme systems for this. Best known is the Angiotensin-converting enzyme (ACE), which converts inactive angiotensin I to the systemic vasoconstrictor angiotensin II. Histamine, bradykinin and serotonin are, like acetylcholine, largely inactivated by the pulmonary endothelium in one passage through the lungs.

3.1 PRODUCTION.

All of the peptide mediators can be and are being synthesized, as are many of the lipid-based mediators. Most of these are available, commercially, for both human and veterinary use (174,178). Recent advances in production technology now enable kilogram quantities per batch of small peptides (10 to 15 amino acids), whereas a few years ago, only milligram quantities would have been possible (165). With larger peptides, not only is synthesis more difficult and cumbersome, but also, complicating secondary and tertiary structure problems may affect activity (165). Most, if not all, of the peptide mediators may be obtained by recombinant DNA (rDNA) technology (180). The lipid mediators require the participation of numerous biosynthetic enzymes and are much less feasible for rDNA technology production.

3.1.1 Biosynthesis.

In the natural synthesis of many of the peptide mediators, peptides are synthesized as portions of larger precursor polypeptides (165,176). It is quite common for there to be more than one neuropeptide contained in a given precursor and for more than one copy of a peptide to be included in its sequence (186, 187). Nearly all peptide precursors include a hydrophobic leader sequence of 15-30 amino acids called a signal peptide (192). In nearly all precursors, the biologically active peptide sequences are bounded on either side by pairs of basic amino acids that provide cleavage signal for trypsin-like processing enzymes (192). The processing enzymes are different for each precursor. In addition, different enzymes may process the same precursor in different ways to produce a preponderance of one or another product. Peptide biosynthesis also entails other forms of post-translational processing (176). Amidation of the C-terminus of the molecule is common in neuropeptides (176). Acetylation of the N-terminus of peptides is also common and in some cases is essential for biological activity (176). Thus, while biosynthesis is possible, for large scale synthesis, additional difficulties for final production in active forms will be encountered (176).

Newer mass production technologies wherein the intact plant or animal organism is used to produce the desired product, e.g., insulin production in the milk from cows, could potentially readily overcome these difficulties (74a,165,176). Indeed, many

in the pharmaceutical industry see the lactating cow as a vast continuous replenishable production vat for many future mammalian products (176).

Specific cytokines, as the demand for their use has risen, are produced in cultured cells using rDNA technology (176). Many of these have or are being tested in humans in selected therapeutic applications (177). Veterinary application of the use of cytokines (as is true for many pharmaceuticals) generally precede human use by years (Spertzel, personal communication).

3.1.2 Chemical Synthesis.

Almost all natural eicosanoids have now been chemically synthesized, including a large number of analogs (174,178). All of the prostaglandins can be made synthetically by a variety of well established synthetic procedures (174,188,189). Modifications are possible and a plethora of synthetic analogs have been designed (188,189). This has resulted in compounds with desirable properties of solubility, increased stability against metabolic degradation, and selective biological activity (176).

Thromboxane A₂ is chemically unstable and difficult to synthesize, but it has been accomplished (188,189). Several synthetic analogs of TXA₂ with enhanced stability have been synthesized and some of them possess potent biologic actions (188,189). TXB₂, the stable metabolite of TXA₂, has been synthesized by several groups (178,188,189).

Prostacyclin (PGI₂) and many synthetic analogs have been synthesized (188,189). The great potential of PGI₂-like compounds as antihypertensive, anti-asthmatic, or anti-thrombotic agents has been the catalyst for extensive effort in this area, resulting in a wide range of synthetic materials.

Because of their great biological importance, and the difficulty in isolating the leukotrienes in quantity from natural sources, a considerable chemical effort has been carried out to synthesize these compounds (177). Thus, almost all of the leukotrienes as well as many stereochemical and structural analogs have been synthesized. Indeed, the complete structural elucidation of leukotrienes was realized by comparison with various synthetic substances which were unambiguously synthesized (188,189). Of all the products derived from the lipoxygenase metabolism of AA, the peptido-leukotrienes have attracted the greatest attention biologically and chemically. During the course of synthesis of the various leukotrienes, a number of closely related analogs have also been prepared (190,191). Because of the promising therapeutic potential of these compounds, like the effort with the prostaglandins, much

attention has and is being given to the development of chemically and biologically stabilized compounds (177).

3.2 STABILITY.

The mediators considered in this paper are either peptides or lipid derived compounds that are relatively stable under reasonable storage conditions. Extended shelf-life needs for animal and human pharmaceutical applications of these products have driven the research for storage stability. Stability under conditions for offensive use is unknown. Stability as discussed in this section is related to their metabolic stability in the body.

3.2.1 Natural Occurring Mediators.

The natural mammalian peptide mediators are generally considered to consist of L-amino acid residues and are readily metabolized (180,181,185,186,187,188,189,190,191,192). While mediators are discussed in terms of fast acting or slow acting (still a matter of minutes rather than hours), this distinction is related more to the generation of additional mediators or other intermediary substances that may account for the full effect of the observed response. Duration of the observed response in turn is related to the regeneration of the mediator as a result of continued stimulus (185,192).

The lipid derived mediators also are readily metabolized to inactive compounds or in some cases to new mediators that may have the counter effect of the original mediator (185,188,189). One passage through the circulation of the lungs removes >60% of iv administered prostaglandins or leukotrienes (185). Greater than 95% of PGE₂ and PGF_{2α} entering the lung are extracted from the circulation in a single pass (185).

The opioid peptides, dermorphin and deltorphin, are considered to be the most potent and selective mediators for the μ - and δ -opioid receptors, respectively (87,88,90,151). As cited in section 3.2.3.4, these are natural opioid peptides albeit from non-mammalian sources (evidence is emerging that these compounds may exist in some mammalian brains as well) (66,68,88,89). Extensive studies have been conducted to synthesize and test shorter and longer derivatives of these peptides, to substitute D-Arg for the D-Ala in dermorphin and to substitute amino acid by amino acid seeking a more stable or more selective peptide, etc (22,23,27,61,66,76,78,91,97,98,127,128,129,131). In the process, peptides with stronger affinity for the receptor(s) or equal (or more) metabolic stability were generated, but none with a significant increased potency over that of dermorphin, a naturally occurring peptide. Analogs of dermorphin are seen as having potential therapeutic analgesic applications.

3.2.2 Biostabilized Compounds.

It is generally assumed that mammalian peptides can be biostabilized by the substitution of a D-amino acid into the moiety, since such substitutions are most uncommon in mammals (165). Indeed, the potency of the opioid peptides, dermorphin and deltorphin, are attributed primarily to the presence of D-Ala and D-Met, respectively, in these heptapeptides (22,23,78,87). Would such D-amino acid substitution metabolically stabilize other mediators such as endothelin, thereby increasing their potency? Such studies have not been reported and unless a biomedical need arises for such a stable compound, such studies are unlikely to be conducted. Substitution, one at a time, of the D-for L-amino acid in neurotensin gave peptides that varied from 0 potency to 1000 times more potent than naturally occurring neurotensin (165).

No comparable example to the dermorphin story is available for the lipid-derived mediators in terms of stabilizing the mediator. However, an antagonist for PAF, that competes for the same receptors, both natural and synthetic, has been reported (167). These studies are directed toward preventing the untoward effects of the natural overproduction of PAF under specific conditions and have not been directed toward compounds that would increase the potency as seen with PAF. It seems reasonable to believe that stable, potent agonists for PAF and perhaps the other lipid mediators could be synthesized. Synthetic PG analogs have been developed with the intention of introducing more specific and potent biological properties than those of the natural PGs (188,189). Of particular importance has been the development of analogs resistant to those enzymes which metabolize and inactivate PGs (176).

Because chemical and metabolic stability is such an important consideration in developing therapeutically useful compounds, a great deal of effort is expended by governments and industry to develop such stable analogs of these natural products (165). For example, there are now a plethora of PGs that can be administered orally which means that not only are these substances able to withstand the rigors of passage through the stomach, but also that they can be absorbed or transported into the circulation to reach the targeted tissue (174,177,178). Another consideration that drives the development of "novel" (and therapeutic) compounds by the industrial medicinal chemists is the necessity for structural novelty in the patent sense (176). (This same need is behind the concerns of proprietary infringement resulting from treaty monitoring of these and related compounds.)

3.2.3 Molecular Substitutions.

Minor differences in amino acid composition, sometimes involving only one or two amino acids, results in marked changes in physiological activity. For example, removing the terminal tryptophan residue of ET-1 reduces its constrictor activity by a factor of approximately ten thousand; removal of the terminal 5 amino acids results in a total loss of activity; and cleavage of the lysine residue at position 9 causes a threefold loss of activity (181).

The endogenous mediators owe their contribution to homeostasis to their rather transient life (metabolic instability) in the circulation (185,186,187); if this stability were altered not only might the effective dose be greatly reduced but also the duration of the effect might be greatly prolonged. For some compounds, e.g., neurotensin, where stabilized forms were sought, increases in effectiveness in in vitro and animal studies up to 1000 times the parent compound have been reported (165). On the other hand for other compounds, e.g., dermorphin, in spite of diligent attempts, no increase in effectiveness was noted (see 3.2.2). Substitution with selected amino acids may increase, decrease or abolish the potency of a mediator; substitution of amino acids may also induce a response that is different than the parent compound (apparently by an affinity for different receptors); even the substitution of a D-amino acid for an L-amino acid may change the affinity for receptors, e.g., substituting D- for L-Cys₄ in somatostatin altered its selective inhibition of insulin to inhibition of glucagon (176). Most substitutions are deleterious to biologic activity.

3.3 ASSAY.

Bioassays, GC/MS, radioassays and ELISAs have been used to measure LTs in tissue fluids with a high degree of sensitivity. Each have a role to play in research and medicine related to these important mediators (9,170,179,192). Because of the importance of the bio-mediators in medicine, standardized assay tests and methodologies are available (192). The difficulty that could be encountered would be in recognizing and assaying "aberrant or unusual" mediators, in quantitating for excessive levels of mediators, or determining exogenous delivery of the mediators. Also, no assay methodologies have been reported for the detection of biomediators in air.

3.3.1 Physiologic/Homeopathic Levels.

The availability of a specific antibody to the low molecular weight compounds can be utilized for the development of sensitive and specific immunological procedures such as radioimmunoassay (RIA) and enzyme-linked immunoabsorbent assay (ELISA) for

detection of minute amounts of antigens or antibodies (192). In addition, the antibodies are useful in examining other areas of study such as tissue distribution, synthesis and in some instances, structure and function of the haptenic molecule. Commercial kits are available for radioassays of most of the mediators (174,178). Eicosanoids generally occur only in minute amounts in biological material, which necessitates the use of very sensitive and accurate quantification methods (188,189). Many immunological methods display satisfactory detection limits for eicosanoid assay, permitting the analysis of even small biological samples, such as sub-milliliter volumes of plasma.

Except for the highly polar peptido-leukotrienes and the unstable epoxy acid LTA₄, lipoxygenase products can be extracted from biological media using classical organic solvent extraction procedures (188,189,190,191). The peptido-leukotrienes are extracted by adsorption on hydrophobic polymers. Extraction procedures for all of the eicosanoids have been published.

3.3.2 Pathologic Levels.

Because of very rapid and efficient metabolism, only very small concentrations of any one eicosanoid are present in the circulation (173,185,192). Therefore, it is extremely difficult to measure the miniscule concentrations in the circulation as an index of endogenous production of these compounds (192). However, circulating concentrations of metabolites are present in greater amounts and quantification of metabolites methodologically may be a more accurate means to assess endogenous production (185). Assays for quantitative measurements of deliberately introduced excess quantities of mediators have not been reported. Unless the mediator was chemically or structurally different than the natural mediator, determination of exogenous versus endogenous production of the mediator could be very difficult.

3.4 MEDICAL/THERAPEUTIC APPLICATIONS.

Because the mediators are part of the natural physiologic functioning of animals and man, they are used extensively in veterinary and human medicine. Indeed, a whole line of the pharmaceutical industry has developed around the production of peptide and lipid mediators for animal and human use. Much of the research on novel compounds, because of legal, regulatory and metabolic requirements, is being driven by this industry.

Cytokines are used or are being considered for use against a variety of neoplastic, infectious, allergic and pathophysiologic conditions (177). Selected leukotrienes and prostaglandins are being considered in treatment of asthmatics and adult respiratory distress syndrome (ironically to counter the action of other

endogenously produced leukotrienes and prostaglandins) (188,189). Prostaglandins are being used in a variety of normal and abnormal physiological applications, e.g., $\text{PGF}_{2\alpha}$, PGE_2 and analogs are used to induce parturition and to terminate undesired pregnancies, PGE_1 and PGE_2 are utilized in delaying closure of the ductus arteriosus in infants born with certain cardiac abnormalities, and PGI_2 has been used in cardiopulmonary bypass operations (188,189,192).

Peptide hormones are used extensively in the animal industry, e.g., milk and meat production, to stimulate increased growth, to maintain pregnancy and to terminate pregnancy, etc. In human medicine peptide hormones are also used extensively, e.g., insulin in the treatment of diabetes, various growth factors to correct metabolic and physiologic deficiencies, etc (Spertzel, personal communication).

3.5 TECHNOLOGY AND THE UNKNOWN.

The rapid advances in molecular genetics have allowed the isolation and cloning of practically any gene from the genome of an organism (74a). These gene-cloning techniques, combined with the ability to express cloned genes in cells growing in culture, enabled the production of useful quantities of specific peptide or protein products for vaccines and replacement therapeutics in such diverse categories as hormones, enzymes, immunomodulators, serum proteins, and viral antigens (74a). Included in this new breed of biological drugs are many endogenous mediators such as TNF, IL-1, growth factors and interferons (177). Little is known about the effects of such novel compounds at higher than physiological levels or when delivered by different methods. Most of them are autocrine and/or paracrine substances, released and exerting their effects only on certain cells and tissues (172,173,192). These substances rarely circulate in plasma, so that tests based on systemic injections probably do not give realistic pictures of their normal, physiological functions and tests based on injections into discrete tissues do not give realistic pictures of the effects resulting from the systemic or aerosol administration of excessive dosages of exogenous mediators.

3.5.1 Aerosol Administration - Known.

Because of the importance of the lipid mediators to asthma and other respiratory distress diseases, extensive studies have been conducted on the effects of these mediators on the pulmonary vascular bed and pulmonary function in vitro and in vivo after intravenous and inhalation challenge (19,40,60,63,101,108,135,140,150). In addition more limited studies have been conducted on the effects of inhalation of aerosols of endothelins, Substance P, and Neurokinin A (44,47,51,71,73).

3.5.1.1 Lipid mediators. In vivo studies in humans have been performed in both normal subjects and asthmatic patients, showing that LTC₄ and LTD₄ are more potent bronchoconstrictors than is LTE₄, whereas LTE₄ causes longer lasting contractions (19,60,101,108,135). The onset of bronchoconstrictive effects in humans are seen more rapidly for LTD₄ and LTE₄ (4-6 min) than for LTC₄ (10-20 min). Contradictory results as to relative potency of the various LTs have been reported dependent on the parameters measured. All, however, are effective at physiologic levels ($\sim 4 \times 10^{-6}$ M) in inducing asthmatic-like small airways contraction in humans. This correlates very closely with animal data.

PGD₂ has potent bronchial smooth muscle activity in vitro and is a potent bronchoconstrictor in vivo after inhalation in either normal or asthmatic subjects (188,189). In vivo PGD₂ has greater bronchoconstrictor activity when compared with either PGF_{2 α} or histamine.

PAF inhalation markedly increases bronchial hyperreactivity for prolonged periods (150). In addition, PAF induces pulmonary vasoconstriction, increases systemic vascular permeability, and stimulates bronchial mucus secretion (7,41,62,63,116). Aerosol challenge of pigs with PAF induced pulmonary obstruction and vasodilation in both the bronchial and nasal circulation, lasting for 15-30 minutes (7). Procedures employed were not optimal for aerosol production and particle size, and the doses administered therefore were relatively large (0.5 mg). The data was sufficient to support the pulmonary effects of systemically administered PAF. Bradykinin challenge under the same conditions (1 mg) induced a similar effect (7). Intravenous administration of these same mediators were given in terms of mediator per kg body weight, suggesting the uncertainty of the aerosol administered dose. In another study 80 μ g exposure induced similar effects in humans with maximal bronchoconstriction observed 305 minutes postchallenge (7). The action of PAF in pulmonary constriction and vasodilation may be through the induction of other mediators such as the peptido-leukotrienes (116,150).

3.5.1.2 Endothelins. In vivo studies in animals have shown that inhalation of ET-1 aerosol induces a potent bronchoconstriction without significant changes in blood pressure (51,71,80,112). ET-1 is more effective than ET-3 or VIC (44,70,71,112).

3.5.1.3 Tachykinins. Neurokinin A exerts a potent contractile action on guinea pig bronchial smooth muscles both in vitro and in vivo (25,95). In addition NKA appears to be particularly active in human isolated bronchi (95). Both NKA and Substance P have been shown to be potent bronchoconstrictors when administered by inhalation (2). This action of these tachykinins are prevented by drugs that attenuate mast cell activation and

inhibit non-cholinergic neural reflexes. Thus, the bronchoconstrictive effects of these tachykinins are via indirect pathways (25,95,185). Other studies have suggested a direct effect of these mediators on small airways smooth muscle cells to account for the bronchoconstriction observed (185).

3.5.2 Aerosol Data - Unknown.

For most of the mediators, little or nothing is known as to the type and degree of effects, when they are administered by aerosol to the lung. Are the larger molecular weight mediators such as IL-1 and insulin capable of transiting the "lung-blood barrier" to exert their effect or are they capable of having a direct effect due to receptors in the lungs? Insulin, in one study, was shown to be as effective by aerosol (in the presence of glycerol or fusidic acid as a vehicle) as by iv in inducing hypoglycemia in rats (Wannemacher, personal communication).

Likewise, little or nothing is known about the administration of doses that are much larger than levels that may be present under natural conditions. The action of many of these mediators have been shown to be different at different levels, e.g., endothelin-1 may induce vasodilation or vasoconstriction depending on dose (iv) administered (16,44,51,59,69,71). Thus, lethality data in animals or humans for most of these mediators are unknown. Certainly those mediators that can cause severe asthma-like symptoms might be expected to be lethal provided their action could be sustained (by continued exposure or by stabilizing the mediator against metabolic degradation). Such data has not been reported.

SECTION 4

DISCUSSION

The endogenous mediators do not meet the criteria for inclusion under Schedule 1 or 2 Chemicals in the CWC text: they were not prior-weaponized; they are not precursors of such agents; they do not have high potential for weapons due to toxicity; and they all have widespread legitimate use in research and medicine. Inclusion in Schedule 3 would have no impact on the legitimate use of these compounds.

But, the impact on peaceful uses of these substances is not the issue that concerns many people. As stated in the paragraph above, the impact on peaceful use should be negligible. Rather, the concern deals with the detection and monitoring of illicit use of these substances. While there is no scientific verification of the development of metabolically stabilized mediators that can have severe incapacitating or lethal effects when delivered by aerosol, this is theoretically within scientific limits of today's technology. Monitoring for such research and development efforts will be difficult and need to be very intrusive, if it is to have any chance to succeed. Assay procedures have been reported for the mediators, and, unlike the toxins, many (perhaps most) of the procedures are standardized because of their use in medicine. The procedures are based on levels of mediators from biological samples; they are not designed for remote, stand-off detection (nor are they designed to differentiate between excessive exogenous or endogenous sources of these mediators). The endogenous mediators, like the toxins, neither emit nor leave a residual telltale signature for detection and identification. Also, the potential permutations and combinations of amino acids, type and length, amidated, acylated, etc., are so vast that definitive proof that a substance exists and has a potential detrimental effect on personnel, may require laboratory verification of the negative effects. An a priori, theoretical, assumption of a particular effect, based on molecular structure, cannot be made for these compounds, e.g., a single amino acid substitution can drastically alter the biological activity and even a D- for an L-amino acid may change the receptor affinity.

Similar, but perhaps less dramatic, arguments can be made for the lipid mediators. For example, there are similar but distinct forms of PAF among animal species and different affinities for receptors suggest that within a single individual there may be different PAF molecules. While most natural antagonists for PAF are based on the same "backbone" structure as PAF, some natural and man-made antagonists have been identified that are structurally different (167). Since such antagonist have been identified, it is theoretically possible that similar, structurally unrelated, agonists for PAF may exist or could be

created. However, because these mediators are all relatively small in size and are dependent for their biologic activity on specific receptors, a degree of limitation exists on potential analogs or homologs that are possible.

SECTION 5

CONCLUSIONS

The endogenous mediators do not meet the criteria for inclusion in Schedules 1 or 2 of Chemicals. Their inclusion in Schedule 3 should have negligible, if any, effect on their legitimate use. Monitoring the improper research, development and use will be difficult if not impossible.

Endogenous mediators delivered by aerosol must transit from the lungs to the circulation and then breach the blood-brain barrier to induce a central effect. To date this has not been accomplished and is not foreseen in the near future. Thus, neuropeptides that act centrally to induce their biologic activity, but are administered by aerosol, are not likely in the near future. The endogenous mediators that need only reach the circulation to induce a cardiovascular effect or those mediators that can induce a direct effect on the respiratory system exist today. Their limitation on biological activity is their rapid clearance from the circulation, much of which occurs in one pass of the circulation through the lungs by clearance mechanisms in the lungs. Any stabilization of this metabolic clearance would markedly alter the degree of biologic activity..

There is a vast array of endogenous mediators that have a natural respiratory smooth muscle constricting activity that can induce asthma-like bronchospasms as well as other features of an asthma attack or an equally dangerous condition known as adult respiratory distress syndrome. These include the lipid mediators (peptido-leukotrienes, PAF, prostaglandins, thromboxanes, and perhaps some others), the endothelins (and sarafotoxins), the cytokines (TNF and IL-1) and perhaps some of the neuromediators or which bronchial smooth muscle receptors exist.

Metabolically stabilized mediators exist in nature, e.g., dermorphin, an opioid peptide with a D-Ala amino acid in its structure is the most potent μ -opioid receptor agonist known. Such naturally stabilized mediators may exist or could be created for the mediators that exert a direct effect on the cardiovascular or respiratory system. Indeed, such man-made stabilized mediators have been experimentally created in the research laboratories for some of the neuromodulators and are constantly being sought for pharmaco-therapeutic applications and in the pursuit of basic knowledge of the mediators and their receptors.

The neuropeptides have traditionally received the greatest attention by the intelligence community and others that are concerned with the potential threat posed by the endogenous mediators; whereas, the lipid mediators have been largely ignored. The emphasis may be misplaced; the neuromediators face

a formidable obstacle to go from air to lung to blood to brain, that, at the least, requires greater dosages for an effect and may not be effective by this route at any dosage. On the other hand, many of the lipid mediators produce a biological effect (asthma like attack) when administered by aerosol at μg or fractions thereof, are readily synthesized chemically, and have many other characteristics that should arouse concern for their inappropriate application.

In summary, the endogenous mediators have widespread use in medicine and are readily available from commercial sources in reasonable quantities. Most of the mediators can be chemically synthesized and/or produced by rDNA technology. Standardized assay procedures are available or published for the known and characterized mediators, but not for the myriad synthetic analogs that are or potentially are available. Nor are procedures established to determine, necessarily, physiologic levels from pathological levels. Nor are detection methodologies available for detection of the mediators in air and, like toxins, the mediators leave no residuum nor signatures.

SECTION 6

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Appendix A

ENDOGENOUS BIOREGULATORS

Living cells produce a wide variety of chemical messengers that are used to communicate with other cells (166,167,168,182,183,184,186,187,188,189,190). These endogenous lipid and peptide mediators include: the cytokines (TNF, interleukins, interferons) (167,168); classical hormones (insulin, ACTH, etc) (171,172,181); eicosanoids (prostaglandins, thromboxanes, leukotrienes, etc) (186,187,188,189); PAF (167); and a number of smaller peptide regulators that act on the CNS, cardiovascular, pulmonary, and other systems (172,173,175,181,182,183,184,186,187,192).

Peptides are a part of the large repertoire of possible chemical mediators for cell-to-cell communication (173). If the source and target cells are at some distance from one another, it is likely that the peptide will be secreted into the blood or other body fluid for transport to its target. In this case the peptide is referred to as a hormone or neurohormone (172,187). If the target cell is in closer proximity to the secretory cell, for example, within the same tissue but not in direct contact, the term parahormone is used to describe the peptide (187). Finally, in the event that the secretory cell lies in close proximity to the target cell, as exemplified by the synaptic arrangement of nervous tissue, the peptide may be termed a neurotransmitter or neuromodulator (187). Peptides synthesized in nervous tissue and having effects on neuronal activity are generally called neuropeptides. Amino acid sequences may be as short as two or three residues in length, as in thyrotropin-releasing hormone, or one to two hundred long as in β -lipotropin, or growth hormone (172). Peptides larger than 50 amino acids are frequently called polypeptides.

In the CNS, alone, more than 40 different chemical substances have been demonstrated or postulated to function as synaptic transmitters (186). One group is comprised of small-molecular weight, rapidly acting transmitters, e.g., acetylcholine. The other group is made up of neuropeptides of much larger molecular weight which are much more slowly acting. The small-molecule, rapidly acting transmitters are the ones that cause most of the acute response of the nervous system, such as transmission of sensory signals to and inside the brain and motor signals to the muscles. The neuropeptides, on the other hand, usually cause more prolonged actions, such as long-term changes in numbers of receptors, long-term closure of certain ion channels, and possibly even long-term changes in number of synapses.

A.1 LIPID MEDIATORS.

Oxygenated derivatives of arachidonic acid (eicosanoids) and platelet-activating factor are lipid mediators produced by cells involved in the mediation of many inflammatory reactions including the mediation of pulmonary inflammation (185,188,189,190,191). Each of the lipid mediators (prostaglandins, thromboxane, leukotrienes and PAF) interact with distinct receptors present on cell surface membranes to induce their biologic, cellular response.

A.1.1 Eicosanoids.

The eicosanoids include the prostaglandins, the thromboxanes, the prostacyclins, and the leukotrienes (185,188,189,190,191). Arachidonic acid is released from cell membranes by a variety of inflammatory stimuli and is oxidized to an array of compounds including the leukotrienes, the prostaglandins, and the thromboxanes (185). These biologically active metabolites have been implicated as critical mediators in inflammatory diseases such as bronchial asthma, arthritis and psoriasis (6,8,18,19,40,48,60,83,92,101,108,109,152). Arachidonic acid is a 20-carbon fatty acid and it is a common constituent of phospholipids in cell membranes. Once arachidonic acid is released from the cell membranes, it may be metabolized via the cyclooxygenase pathway or via the lipoxygenase pathway (137,185). The biotransformation of free arachidonic acid via the enzyme cyclooxygenase leads to the formation of prostaglandin (PG)_{D₂}, PGE₂ and PGF, as well as the formation of thromboxane (TX)_{A₂} and prostacyclin (PGI₂) (185,188,189,190,191). The lipoxygenase pathway leads to the formation of the leukotrienes and an array of eicosatetraenoic acids.

Eicosanoids display a number of biological actions with direct implication to the microvascular bed and the process of inflammation (190,191). Leukotrienes (LTs) as a group account for virtually all leakage of plasma that is not due to liberated histamine (19,92,101,188). The peptido-leukotrienes may be directly responsible for the initial vasoconstriction and LTB₄ for at least part of leukocyte recruitment (19,40,60,108). Vasodilating prostaglandins (PGD₂ and PGI₂) contribute to the dynamics of inflammation (188,189,190,191). Unlike the leukotrienes they seem to function primarily as modulators, with capacity to enhance and inhibit events in that process (8,166,185). Finally the lipoxins represent a new class of eicosanoids with potential to influence inflammation (188,189). The microvascular actions of LXA₄ (vasodilation, inhibition of leukocyte migration, and restriction of leukocyte-dependent extravasation of plasma) imply a mechanism for down-regulation of inflammation (189).

In the normal physiologic situation, the biological actions of AA metabolites are limited to the local site of biosynthesis as opposed to exerting systemic effects (188,189). Therefore, these AA metabolites are generally considered to be local rather than circulating hormones. A major reason that AA metabolites do not exert systemic effects under normal circumstances is that these compounds are rapidly and efficiently metabolized to biologically inactive metabolites (137). Thus, the biologically active compounds are prevented from reaching the systemic circulation in sufficient concentration to exert effects at sites distant to the origin of biosynthesis. In addition, some AA metabolites are chemically very unstable, in particular TXA₂ and PGI₂, so that spontaneous nonenzymatic chemical degradation to biologically inactive compounds may be an additional mechanism which limits the biological actions of these compounds to the local site of formation (167,188,189).

A.1.1.1 Prostaglandins and Thromboxanes. The prostaglandins are a class of 20-carbon, oxygenated, unsaturated acidic substances derived *in vivo* principally from arachidonic acid (188,189). These compounds which are formed widely in mammalian tissues and fluids are intimately associated with a formidable array of physiological processes and the administration of PGs, not unexpectedly, can elicit a complex spectrum of pharmacological responses (166,188,189,190,191). Prostaglandins, like other endogenous mediators, exert their biological effect by interacting with receptors which show selectivity for compounds with a particular geometry (188,189). PGD₂, PGE₂, PGI₂ and TXA₂ are stable active products produced by the action of cyclooxygenases on arachidonic acid.

Prostaglandin D₂ produces a broad range of biological effects such as sleep induction, bronchoconstriction, vasoconstriction, vasodilation, and the inhibition of platelet aggregation (166,188, 189,190,191). Pharmacological analyses have indicated the presence of two groups of PGD₂ sensitive tissues and cells based on the difference in the order of potency of PGD₂ and its analogs (62,63). The action of PGD₂ is believed to be mediated by PGD₂ receptors on the plasma membrane. Platelets, mast cells and intestinal cells appear to have one set of receptors whereas the other set have been demonstrated in the pulmonary and cardiovascular systems (185). The former appears to be PGD₂ specific and the latter appear to respond also to PGF_{2α} (188,189). PGF_{2α} and PGD₂ are bronchoconstrictors which are released under allergic conditions such as asthma (185). Inhalation of these PGs produces bronchoconstriction. PGD₂ is more potent than PGF_{2α} in both asthmatic and non-asthmatic subjects (185). PGD₂ has also been shown to increase nasal congestion without nasal secretion (8,48, 188,189). The long-lasting component of the allergen-induced vasodilation in airways, especially in the nasal mucosa, may be caused by the

release of PGD₂, acting independently of sensory neurons (8,48). Allergen and PAF aerosol challenge in the lung may also induce the release of a vasodilatory prostaglandin, (possibly PGD₂) into the systemic circulation, thereby inducing nasal vasodilation (8,41,48,167).

PGE₂ exhibits vasodilating and smooth muscle dilating activity (188,189). It also has platelet aggregating activity; at high concentrations it inhibits aggregation and at lower concentrations it enhances ADP-induced aggregation (62). PGE₂ has little effect on vascular permeability directly, but appears to potentiate the exudation produced by other inflammatory mediators. This has been attributed to its vasodilator activity. PGE₂ appears to act on at least three receptors (188,189). Greater than 95% of PGE₂ and PGF_{2α} entering the lung are extracted from the circulation in a single pass (185). Collectively > 60% of all lipid mediators are cleared from the circulation in a single pass through the lungs.

Endogenous prostaglandins in the brain, PGD₂ and PGE₂, are probably the ultimate endogenous sleep-regulating substances, PGD₂ inducing sleep and PGE₂ wakefulness. The balance of these two compounds in the sleep and wake centers in the preoptic area and posterior hypothalamus appears to be responsible for maintaining the sleep-wake cycle (190,191).

Prostaglandin synthesis has been shown to be stimulated by a range of mediators including ATP, interleukin-1 and substance P and in parts of the CNS, are involved in regulating nociception in addition to the sleep/wake cycle cited above (173).

A family of hydroxylated, cyclized, poly-allelic fatty acids that lack the prostanoid acid backbone of prostaglandins have been designated thromboxanes (185,188,189). TXAs are bicyclic and are derived from prostaglandin endoperoxides. TXAs are highly unstable in aqueous conditions, but may be partially stabilized by plasma constituents. TXBs are stable products derived spontaneously by hydroxylation of TXAs.

TXA₂ and PGI₂ have opposing biologic activities and the balance between these two mediators is thought to be critical for pulmonary homeostasis (185). Whereas TXA₂ is a potent bronchoconstrictor, pulmonary vasoconstrictor and platelet aggregator, PGI₂ is a bronchodilator, vasodilator and inhibitor of platelet aggregation (185).

A.1.1.2. Leukotrienes, Lipoxins, etc. In response to various stimuli including other mediators, e.g., PAF, membrane-bound arachidonic acid is released and metabolized by lipoxygenases (for cyclooxygenase metabolism see A.1.1.1 above) with the

formation of leukotrienes, lipoxin and various other newly identified but incompletely described products (150,188,189).

The leukotrienes are potent mediators generated by almost all cell types involved in inflammatory reactions (92). Leukotrienes occur in two structural subtypes, differing with regard to the presence or absence of a peptide side chain (54). Arachidonic acid is metabolized via 5-lipoxygenase to LTA₄ and subsequently to either LTB₄ or to the peptido-leukotrienes (LTC₄, LTD₄, LTE₄) (188, 189). The peptido-leukotrienes are also termed sulphidopeptide leukotrienes or cysteinyl leukotrienes because each contains a thioether-linked peptide. Many cell types are capable of this synthesis; it occurs in the brain as well as other tissues throughout the body (54,188,189).

In the brain LTC₄ is conceivably the most important (54,186). Its action has been linked to both physiological (control of neural excitability, neuroendocrine function) and pathophysiological (cerebral ischemia, cellular and vascular edema) events. Whereas, in the respiratory system LTD₄ and LTE₄ appear to be more important (185,187). LTB₄ is a potent chemo-attractant for inflammatory cells and may play a role in the late phase of inflammation commonly seen in asthmatics, whereas LTC₄, LTD₄ and LTE₄, collectively identified with the classical slow-reacting substance of anaphylaxis, SRS-A, have potent, acute pharmacological effects such as smooth muscle contraction, stimulation of bronchial mucus secretion, and provocation of increases in vascular permeability which make them potential mediators of allergic symptoms (19,40,92, 101,108,135).

Leukotriene B₄ has been reported to have various pharmacological actions such as: neutrophil chemotaxis, aggregation, and degranulation; enhanced vascular permeability; and a bronchoconstrictor activity via an indirect mechanism involving stimulation of other leukotrienes (188,189). High levels of LTB₄ are detected in lesions of human inflammatory disease, for example, psoriasis, gout, rheumatoid, colitis and myocardial ischemia.

The sulphidopeptide leukotrienes exhibit a diverse spectrum of pathophysiological effects that may be important in a variety of inflammatory conditions (188,189). Sulphidopeptide leukotrienes have been demonstrated to be potent vasoconstrictors of coronary, mesenteric, pulmonary and renal vasculature (186, 187). They also induce increases in vascular permeability, and produce bronchoconstriction and myocardial depression (92,101, 108,135). These leukotrienes also have been shown to alter cardiovascular function when administered to animals (29,163). The general profile of cardiovascular alterations is similar to that seen with endotoxins, suggesting that the leukotrienes might be important mediators of septic shock (19,29,135,163,188,189).

The role of sulphidopeptide leukotrienes in airway muscle contraction has been investigated in a wide number of studies, both in vitro and in vivo, and in animal and in human model systems (19, 29, 40, 60, 108, 135, 150, 163, 188, 189). In humans LTC₄ is at least 1000 times more potent than histamine in causing muscle contraction in bronchi and trachea and LTD₄ presents a potency very similar to LTC₄ (54, 108). In vivo studies in humans have confirmed that LTs are a potent stimulation of airways smooth muscle (54). LTC₄ and LTD₄ are more potent than LTE₄, whereas LTE₄ causes longer lasting contractions (108). The onset of bronchoconstrictive effects are seen more rapidly for LTD₄ and LTE₄ (4 to 6 min) than for LTC₄ (10 to 20 min) supporting the theory that LTC₄ is metabolized to LTD₄ before it can exert its maximal effect (108).

The peptido-leukotrienes LTC₄ and LTD₄ are potent bronchoconstrictors (108, 188, 189). Asthmatic patients are hyperresponsive to bronchial inhalation of LTC₄ or LTD₄ compared with histamine (108). The biologic actions of LTC₄ in tissues are mediated by a receptor distinct from LTD₄ and LTE₄, which appear to share a common receptor(s) (185). LTD₄ induces hydrostatic pulmonary edema (101). On a molar basis, LTC₄ and LTD₄ have much greater activity than do prostaglandins, PGD₂, PGE₁, PG_{2α}, PGI₂, or histamines in the induction of airway glycoprotein secretion (92).

Most inhalation studies have been done with LTD₄ because it is more stable than the other two leukotrienes (108). PGD₂ has potent bronchial smooth muscle activity in vitro and is a potent bronchoconstrictor in vivo after inhalation in either normal or asthmatic subjects (48, 188, 189). In vivo PGD₂ has greater bronchoconstrictor activity when compared with either PGF_{2α} or histamine. PGD₂ can act as either a pulmonary vasoconstrictor or as a systemic vasodilator, e.g., causes flushing and nasal congestion (48, 188, 189).

Studies on the key role of the peptide side chain towards the recognition binding site of LTD₄ and its effects on biological activity revealed that the replacement of the peptide part by other peptides, amino acids or amines resulted in a 1-3 orders of magnitude decrease of the smooth muscle contractile activity (99). However the amino group of LTD₄ was not critical for the contractile activity (99). Studies are progressing for a more stable and equally potent analog for LTD₄.

Lipoxins are the most recently discovered class of eicosanoid which potentially may be of importance since they exhibit a range of biological activities distinct to those of the related leukotrienes, prostaglandins and thromboxane (188, 189). Originally two main forms were characterized and given the trivial names lipoxin A₄ and lipoxin B₄. Subsequently a large

number of isomers of LXA₄ and LXB₄ have also been reported and more recently lipoxins LXC₄, LXD₄ and LXE₄ have been identified; they are formed in a cascade in a homologous way to the peptido-leukotrienes. Despite the great interest in lipoxins, such compounds have proven to be problematic in studies on their synthesis and identification.

A.1.2 PAF.

PAF is a potent phospholipid, autacoid mediator implicated in a diverse range of human pathologies including shock, ischemia, cardiac and systemic anaphylaxis, CNS and renal disorders, asthma, and a variety of inflammatory conditions (107,110,143,167). A range of molecular species of PAF has been reported but it is not known if these act upon the same or different receptors.

Biological activity of PAF is dependent on the presence of ether-linked alkyl groups (65,155,167). PAF is rapidly degraded by a wide variety of cell types and enzymes (1,155). PAF has a profound influence on the cardiovascular system, involving a range of direct and indirect effects on the heart, blood vessels, and microvasculature (52,53,62,155,167). The most obvious effect of PAF is a dose dependent fall in arterial blood pressure by relaxation of vascular tone, although a reduction in circulating volume due to extravasation of plasma is a contributing factor (155).

Indirect effects of PAF on the circulation are secondary to the cellular release of potent vasoactive substances such as the prostaglandins, thromboxane from platelets and the leukotrienes from leukocytes (155). In fact, the biosynthesis and actions of PAF are closely related in many conditions to the cyclooxygenase and lipoxygenase products of arachidonic acid. PAF and these metabolites are jointly formed, have overlapping and synergistic activities and arachidonic acid release is stimulated by PAF which in turn enhances the production of these eicosanoids (155).

A.1.3 Lipid Mediators in Asthma.

Many kinds of chemical mediators have been considered to be involved in the pathophysiology of bronchial asthma (40,172,173). The airflow obstruction in bronchial asthma is attributable to four major components: swelling of the airway wall, increased inflammatory cells in the airway walls, increased luminal secretions and muscle contraction (108). Histamine, LTs, substance P and selected other compounds are able to stimulate the increased vascular permeability after intradermal or topical application (108).

The peptido-leukotrienes elicit various features of asthma such as bronchoconstriction, mucus hypersecretion, an increase in vascular permeability and an increase in bronchial responsiveness to histamines (19,108,188,189). LTs have been shown to exert this effect when administered by aerosol (19,101). Inhalation of LTC₄ and LTD₄ causes bronchoconstriction in both normal and asthmatic individuals (19,101,108,135). Given increasing concentrations of inhaled LTD₄, all subjects showed progressive decreases in airflow (19,60,108). LTD₄ has been shown to increase nasal congestion (but not secretions) as well (101). LTD₄ and PAF were shown to cause increased microvascular leakage throughout the respiratory tract, unlike histamine which failed to cause a significant increase extravasation in the pulmonary airways (101,150). PAF was shown to exert the most potent activity (150).

Administration of LTD₄ as well as 5-, 12-, and 15-HETE, LTE₄, significantly increased tracheal mucous gel layer secretion in a dose dependent manner (108). LTC₄ and LTD₄ produce dose-related increases in mucus production at a concentration of 1 to 1000 picograms with a somewhat higher potency for LTD₄ than for LTC₄ (108). LTD₄ is the most potent stimulant of human airway mucus secretion that has been studied (108).

Thus, sulphidopeptide leukotrienes are capable of inducing all three processes of asthma; edema, mucus secretion and muscle contraction (108). Their potencies in this induction suggest that they may be of paramount importance.

LTB₄ has many important functions relevant to the mediation of airway inflammation (48,92,135). LTB₄ stimulates leukocyte chemotaxis, chemokinesis, aggregation, degranulation, and adherence to vascular endothelium (48). Installation of LTB₄ into the airways promotes the influx of functionally active neutrophils into the lungs (48).

PAF appears to be a modulator rather than a mediator in airway hyperactivity (63,167). PAF induces bronchoconstriction directly by action on airway smooth muscle PAF receptors and indirectly by induction of other bronchoconstrictor compounds such as TXA₂, peptido-leukotrienes and complement anaphylaxins (140,150). PAF inhalation markedly increases bronchial hyperreactivity for prolonged periods (140). In addition, PAF induces pulmonary vasoconstriction, increases systemic vascular permeability, and stimulates bronchial mucus secretion (63).

Thromboxane A₂ is a potent bronchoconstrictor (40,83,109,150). Secondary production of TXA₂ is stimulated by LTC₄ and LTD₄ (40,101). Bronchoconstriction induced by inhaled LTC₄ can be inhibited by TXA₂ antagonists (40).

A.2 NEUROPEPTIDES.

In the past few years a growing number of neuropeptides have been identified in the CNS (162,180,182,183,184,187,192). Neuropeptides are present in all major brain areas (186,187). Many are also present in the peripheral nervous system and non-neuronal tissues like the pituitary gland, adrenal gland, gastrointestinal mucosal cells, and the pancreas (186,187). Several neuropeptides were originally characterized in non-neuronal cells and have been reported to be present in the CNS (172). For convenience, the neuropeptides, in this report, have been arbitrarily classified into five groups.

The neuropeptides are synthesized as integral parts of large protein molecules by the ribosomes in the neuronal cell body (186,187). The original protein is enzymatically split into smaller fragments and thereby release either the neuropeptide itself or a precursor of it. The neuropeptide is then packaged into minute vesicles that are released into the cytoplasm and transported to the tips of the nerve fibers by axonal streaming of the axona-cytoplasm. The Vesicles release their transmitter in response to action potentials in the same manner as for small-molecule transmitters. Much smaller quantities of these transmitters are released than for the small-molecule transmitters which is partly compensated for by the fact that the neuropeptides are generally a thousand or more times as potent as the small-molecule transmitters. Also the neuropeptides usually cause much more prolonged actions. Some of these actions include prolonged closure of calcium pores, prolonged changes in the metabolic machinery of cells, prolonged changes in activation or deactivation of specific genes in the cell nucleus, and prolonged alterations in numbers of excitatory or inhibitory receptors. Some of these effects can last for days or perhaps even months or years. Our knowledge of the neuropeptides is only at an early beginning (172,173,186,187).

A.2.1 Hypothalamic Neuropeptides.

These peptides are present in highest concentration in the hypothalamus and are involved in regulating pituitary function (releasing or release inhibiting hormones) or in acting as neurohormones on the periphery (posterior pituitary hormones) (172,182). They are also widely distributed in other parts of the brain and may act as neurotransmitters at extrahypothalamic sites. These neuropeptides include: luteinizing hormone-releasing hormone; thyrotropin-releasing hormone; corticotropin-releasing hormone; growth hormone-releasing factor; somatostatin; vasopressin; and oxytocin.

A.2.1.1 Somatostatin. The cyclic peptide somatostatin (SS) was first described in extracts of hypothalamus and was discovered during the search for a growth hormone-releasing factor

(184,187). This peptide is present in brain regions outside the hypothalamus as well as in peripheral tissues (172,184,187). By icv administration, SS can elicit marked behavioral arousal, hyperkinesia, muscle tremor, and rigidity and seizures (172).

A.2.1.2 Vasopressin, Oxytocin and Related Peptides. Both vasopressin and oxytocin are nonapeptides; both have a cysteine bridge at the 1-6 position (172,182). Most sub-mammalian vertebrates have only one such peptide, arginine vasotocin, which is also found in mammalian pineal glands and which differs from oxytocin and vasopressin in only one amino acid. Vasopressin possess minimal oxytocic activity and oxytocin possess minimal antidiuretic activity (182). Oxytocin is less widely distributed in the brain than is vasopressin.

Vasopressin is a potent vasoconstrictor, but when it is injected in normal individuals, there is a compensatory decrease in cardiac output, so that there is little change in blood pressure (182,186,187). In large doses vasopressin raises blood pressure through vasoconstriction (182,187). Vasopressin and related peptides have been shown experimentally in rats to affect the learning and memory processes (182,187). More recent studies have uncovered a multitude of central effects ranging from brain development to maternal behavior, from temperature to cardiovascular regulation, from sexual behavior to drug seeking behavior (180, 182,187).

The brain is capable of generating highly selective fragments from both oxytocin and vasopressin (182). These fragments may be the endogenous ligands for the memory and cardiovascular regulating effects of vasopressin and oxytocin. Binding sites for some of these fragments have been found and they are different from vasopressin and oxytocin binding sites in the brain (182). Fragments of the parent hormones are highly active and in some cases even more so than the parent hormone. In contrast thermal regulation is a function of the whole parent molecule (182).

A.2.2 Pituitary Peptides.

This group is constituted of neuropeptides which are characteristically synthesized by anterior pituitary cells, but several neuronal cell groups in the brain are also able to synthesize them (182). Their normal concentrations in the brain are one or two orders of magnitude lower than in the pituitary. These neuropeptides include: luteinizing hormone; thyrotropin; growth hormone; prolactin; corticotropin and melanocyte-stimulating hormones, α and β .

A.2.3. Opioid Peptides.

There are three families of opioid peptides in the brain: endorphins, enkephalins, and dynorphins (172,182,187). All of them are derivatives of three large precursor molecules: pro-opiomelanocortin, pre-proenkephalin, and pre-dynorphin/neo-endorphin, respectively (182). Opioid peptides are widely, but rather individually, distributed in the CNS and in the GI (182). Opioid receptors are present in the brain, GI and lungs (160,186, 187). Opioid peptides have been isolated and identified from non-mammalian sources as well, e.g., dermorphin and deltorphin from frogs (122).

The brain and the gastrointestinal tract contain receptors that bind morphine. Endogenous ligands for these receptors are called opioid peptides (186,187). Two closely related pentapeptides called enkephalins are found in nerve endings in the GI tract and many different parts of the brain; they appear to function as synaptic transmitters (172). They have analgesic activity and decrease intestinal motility.

At least 20 active opioid peptides have been identified in mammalian tissues (88). Unlike other peptides, however, the opioid peptides have a number of different precursors. Each has a pre-pro form and a pro form from which the signal peptide has been cleaved (172,182). Proenkephalin was first identified in the adrenal medulla, but it is also the precursor for met- and leu-enkephalin in the brain (172). Each proenkephalin molecule contains 4 met-enkephalins, one leu-enkephalin, one octapeptide and one heptapeptide (182).

Pro-opiomelanocortin, a large precursor molecule found in the pituitary gland and the brain, contains β -endorphin, a polypeptide of 31 amino acid residues that has met-enkephalin at its N-terminal (172). Other shorter endorphins may also be produced, and the precursor molecule also produces ACTH and MSHs. There are separate enkephalin-secreting and β -endorphin-secreting systems of neurons in the brain (172). Beta-endorphin is also secreted into the blood stream by the pituitary gland.

A third precursor molecule is prodynorphin, a protein that contains 3 leu-enkephalin residues associated with dynorphin and neoendorphin (172). Dynorphin 1-17 is found in the duodenum and dynorphin 1-8 in the posterior pituitary and hypothalamus. Alpha and β -neoendorphin are also found in the hypothalamus. The reasons for the existence of multiple opioid peptide precursors and for the presence of the peptides in the circulation as well as the brain and the GI tract are presently unknown (172).

A.2.3.1 Endorphins. A 31 amino acid peptide isolated from pituitary extracts contained at its N-terminus the met-enkephalin sequence and was nearly identical to the C-terminal sequence of a

peptide called β -lipotropin (49,187). This peptide was called β -endorphin (β -E) and was isolated along with two other sequences called α - and γ -endorphin. These latter two peptides proved to be the first 16- and 17-amino acid residues of β -E, respectively (180,187). Beta-E was shown to be several times more potent than met-enkephalin and a thousand times more potent than morphine in the assay systems studied (187). Beta-E has been shown to exert a wide variety of CNS-mediated effects following ic administration (187). These include muscular rigidity and immobility (catatonia); sedation; enhanced locomotor activity; seizure activity; behavioral arousal; and effects on blood pressure and body temperature. Beta-E 1-27 (sometimes called C' fragment) also exists in brain tissue but acts as an opiate antagonist rather than as an agonist (187). Both α - and γ -endorphin are only weakly active as opiates but exhibit activities more similar to psychostimulant and neuroleptic drugs, respectively, when administered to animals (187). Acetylation of these fragments does not alter these non-opiate activities. Post-translational processing of β -E can then dramatically alter the activity of the peptide (187). This observation is becoming more common as more is learned about post-translational processing of neuropeptides and the pharmacology and physiology of the fragments produced. Proof of physiologically important peripheral effects of β -E is still lacking (187).

A.2.3.2 Enkephalins. A pentapeptide, Tyr-Gly-Gly-Phe-Met, was the first opiate peptide isolated from brain tissue and was called enkephalin (Met-enkephalin) (187). The precursor pre-proenkephalin molecule contains four copies of met-enkephalin, and single copies of leu-enkephalin, met-enkephalin-Arg⁶-Phe⁷, and met-enkephalin-Arg⁶-Gly⁷-u⁸ (172). Enkephalins are widely distributed in the central nervous system (172,187). Enkephalins are present not only in brain but also in tissues of the gut and in the adrenal medulla (172,187).

A.2.3.3 Dynorphin/ α -neo-endorphin. Dynorphin/ α -neo-endorphin, the third class of opioid peptides, was initially isolated from pituitary tissue (187). The peptide isolated was different from those previously described in that its N-terminus contained the sequence of leu-enkephalin followed by an amino acid sequence entirely different from that of β -E (187). When tested for opiate activity in gut smooth muscle preparations, it was shown to be even more potent than β -E and was given the name dynorphin. Five dynorphin-related peptides, dynorphin A, dynorphin (1-8), dynorphin B, α -neo-endorphin, and β -neo-endorphin, have been mapped in the rat brain (187,192). Their distribution in the CNS is not identical and the ratio of their molar concentration varies from nucleus to nucleus.

A.2.3.4 Dermorphins. In the past few years, a new family of small (7 amino acids) but highly potent endogenous opioid

peptides has emerged (45,46,67,68,87,88,104,106,122,130,133,139,144,145,149,151,161). First, dermorphin was isolated from the skin of arboreal frogs (27). With respect to its analgesic activity, dermorphin is the most potent morphine-like agonist actually known (87). It also combines the highest affinity and selectivity for the μ -opioid receptor (87). Then dermenkephalin, also referred to as deltorphin or dermorphin gene-associated peptide, and deltorphin I and II were isolated from the same source and were found to exhibit an affinity and a selectivity for the δ -opioid receptor which are equal or superior to those of the prototypical δ -ligands (enkephalins) (87,90). Deltorphin is the most powerful and selective natural δ -opioid peptide known (90,151). These heptapeptides are devoid of structural homology with the mammalian opioids: they contain a common N-terminal sequence Tyr-(D-Ala or D--Met)-Phe, and they are exquisitely selective for a single subtype of opioid receptor (87).

Dermorphin is at least 1000-fold more potent than morphine in producing analgesia, catalepsy and respiratory depression (88). In the analgesia tests the N-terminal tetrapeptide analog of dermorphin is even more potent and longer acting than the parent compound (22,23). Studies in animals have confirmed that dermorphin has a potent antinociceptive effect when administered icv, iv, or sc (22,23,61,93,97,126,131,132). In man dermorphin exerts neuroendocrine effects typical of endogenous opioids, increasing prolactin, growth hormone and thyroid stimulating hormone and decreasing ACTH, β -lipotropin, β -endorphin and cortisol levels (66,77). The D-Ala moiety and N-terminal sequence are of crucial importance for the full opioid activities of dermorphin, but the fragments represent rather less potent activities than the parent heptapeptide (61,78).

Biological studies on synthetic dermorphin peptides have given the following results: The activities of shorter N-terminal homologs decrease with a decreasing number of amino acids; the N-terminal tetrapeptide is the minimum sequence required for activity; the three N-terminal residues being of major importance; when Tyr at position five of the dermorphin peptide is substituted by a L or D residues of similar size, aromatic or non-aromatic analogs showing increased activities which occasionally discriminate between μ and δ receptors are obtained (22,23,27,76,78,85,91,97,98,103,125,126,127,128,129,132,146,158).

A.2.4 Brain-Borne Gastrointestinal Hormones.

These peptides are distributed in the gastric and intestinal mucosal cells and pancreatic cells (182). Many of them occur in the nervous system of lower species only, while others exist as native and bioactive neuropeptides in mammalian brains. These neuropeptides include: substance P; neuropeptide Y; vasoactive intestinal polypeptide; peptide HI-27; cholecystokinin; bombesin; neurotensin; secretin; motilin; galanin; glucagon; and insulin.

A.2.4.1 Substance P. Substance P is one member of a family of peptides that has been termed tachykinins (94). Included in this family is a structurally related peptide called substance K (neurokinin A) and neuromedin K (neurokinin B), all of which share the common carboxyl-terminal sequence, Phe-X-Gly-Leu-Met-NH₂ (94,187). Tachykinins share several biological actions in common, including potent smooth muscle contracting and vasodilatory activities, as well as being potent secretagogues. Substance P was the first peptide found in neural tissue and is the best characterized of the various neuropeptides. Tachykinins are widely distributed in both the CNS and peripheral tissues and evoke a variety of biological activities in different tissues (94). The three tachykinins excite neurons, act as potent vasodilators, and contract many smooth muscles. They are distinguished by quantitative differences in their relative potencies of pharmacological activities. These various activities are generated by the existence of diverse types of tachykinin receptors (94). The three tachykinin receptors have distinct affinities for the tachykinins and are distributed differently in various mammalian tissues. Thus, the varied physiological responses of the three tachykinins occur as a result of the selectivity and different distribution of the several types of the receptors (94).

Substance P is found in appreciable quantities in the intestine, where it may be a chemical mediator in the myenteric reflex (172). In the nervous system, it is found in nerve endings in many different locations. Little is known about its synthesis or catabolism. However, it is produced by primary afferent neurons in tissue culture, and it is almost certainly the transmitter released in the substantia gelatinosa by the neurons mediating nociception (pain) (172). Upon sc injection, substance P produces vasodilation, and its release from the peripheral ends of the primary afferent neurons is probably responsible for the axon reflex (2,11). The preprotachykinin gene that codes for substance P can be processed to form two different mRNAs (172). By elimination of one set of introns during posttranscriptional processing, the mRNA for substance P is produced (94). However, in some parts of the nervous system and other tissues, different splicing occurs, with the production of an mRNA that instead produces the related polypeptide substance K (94).

Neurokinin A has been shown to exert a potent contractile action on bronchial smooth muscles both in vitro and in vivo (25). Although this effect seems to be due either to a direct action of this peptide on specific muscular receptors or to an indirect effect on mast cells and/or nerves, its mechanism of action is still unknown. A dose-related severity of bronchospasm can be induced by inhalation of NKA in normal test subjects (25).

Non-mammalian tachykinins have been isolated from the skin of American frogs: physalaemin, the prototype of the amphibian tachykinin peptide family and phyllomedusin (36). Physalaemin belongs, like the Australian cueroleins, to the physalaemin/substance P tachykinin subfamily; phyllomedusin occupies an intermediate position between this subfamily and that of kassinin/eleodoisin (36,182). Some preliminary data suggest that minor amounts of other tachykinins may occur in skin extracts of *P. bicolor*.

A.2.4.2 Neuropeptide Y/Pancreatic Polypeptide Superfamily. All vertebrate members of this regulatory peptide superfamily contain 36 amino acid residues, terminating in either a tyrosine or phenyl-alanine amide (82). The Neuropeptide Y/pancreatic polypeptide superfamily is of widespread occurrence in vertebrates and invertebrates as indicated by numerous immunocytochemical and radioimmunometric studies (82). Pancreatic polypeptide (PP), the first member to be discovered, occurs in a discrete population of endocrine cells within the vertebrate pancreas. Neuropeptide Y occurs within central and peripheral neurons and a subpopulation of adreno-medullary cells, whereas peptide tyrosine tyrosine occurs within mucosal endocrine cells of the distal intestine (82). Neuropeptide Y probably functions as a cotransmitter with norepinephrine. Recently, the first member of this regulatory peptide superfamily has been isolated and sequenced from extracts of a tapeworm (82). This peptide, designated neuropeptide F, is comprised of 39 amino acid residues and terminates in a phenylalanine amide (82). The C-terminal tetra-peptide amide of neuropeptide F is identical with that of amphibian and reptilian PP.

A.2.4.3 Bombesin-like Peptides. Bombesin (a 14 amino acid peptide) was first isolated and characterized from methanol extracts of frog skin (183). Subsequently, bombesin-like activity has been found throughout mammalian intestine, lung, brain, and plasma (169,182). Gastrin-releasing peptide (GRP) has a striking homology to bombesin in the C-terminus region and is considered by many to be mammalian bombesin (169,183).

In general bombesin-like peptides are discretely distributed in the brain and their density is less than that of other peptides such as substance P or neurotensin (183). Bombesin releases gastrin, gastric acid and CCK among other activities.

Alytesin, litorin, and ranatensin are peptides structurally related to bombesin containing 14-, 9-, and 11-amino acid residues that were also found in frog skin and appear to have mammalian counterparts as well (39,183). It is highly probable that identical or closely related molecules also occur in the skin of other American ranid frogs (39). The contents of phyllolitorins and rohdeilitorin varied between 4 and 7 $\mu\text{g/g}$ fresh skin. Ranatensin and rohdeilitorin belong to the

litorin/ranatensin subfamily of the bombesin peptide family; further studies are required to decide whether the phyllolitorins constitute a new subfamily (39).

A.2.4.4 Neurotensin. Neurotensin is present in mammalian gut and distributed throughout the CNS (172,187). The many pharmacological actions attributed to neurotensin include induction of hypotension, increased vascular permeability, hyperglycemia, increased intestinal motility, and inhibition of gastric acid secretion (187).

A.2.4.5 Insulin/insulin-like Growth Factors. Insulin is secreted from the beta cells of the pancreatic islets into the hepatic portal circulation and has important actions on the liver as well as on peripheral tissues (192). Insulin is the dominant glucose regulating hormone and is a potent and critical hormone. Profound insulin deficiency and marked insulin excess can both be lethal. Insulin is necessary for prolactin and other hormonal functions (186,187,192). Its major sites of action are liver, muscle and adipose tissue. However, insulin has also shown distinct or permissive effects in many tissues or systems, including pancreas, kidneys, brain, lung, immune system, platelets, nervous system, and bone. Insulin administered by aerosol, in the presence of lipophilic substances, is as effective as by iv administration to obtain a given effect (Wannemacher, personal communication).

A family of GFs, which bears a close structural similarity to insulin, has mitogenic activity on a wide variety of cell types and has been termed insulin-like growth factors (176,177, 192). There are basically two members of the insulin-like GF family: IGF I (also called somatomedin C) containing 70 amino acids; and IGF II (MSA,) containing 67 amino acids (192). Although insulin, IGF I and IGF II each has their own specific receptor, the structural similarity among the peptides is so great that IGF I cross-reacts weakly with the insulin receptor and hence has weak insulin-like activity; conversely insulin binds to the IGF I receptor and has weak IGF-like activity (192). Also IGF I can interact with the IGF II receptor. However, IGF II does not interact with either the IGF I or the insulin receptors.

Three basic types of biological activity have been reported for IGFs (192). First they stimulate proliferation of a wide variety of cell types. Second, they stimulate incorporation by chondrocytes of sulfate into chondroitin sulfate of cartilage. Third, they have a variety of insulin-like effects such as alterations in the metabolism of adipocytes. Many of the biological effects of growth hormone are mediated by the increased production of IGFs.

A.2.5 Other Neuropeptides.

These peptides are also widely distributed in the CNS, some of them in high concentrations and they also occur in non-neuronal tissues (182). Many neuropeptides in this group are known as strong, vasoactive substances. These neuropeptides include: angiotensin II; FMRFamide; bradykinin; delta sleep-inducing peptide; and atrial natriuretic factor.

A.2.5.1 Angiotensin II. The octapeptide angiotensin II has a generalized vasoconstrictor action (172,182). It is formed from angiotensin I liberated by the action of renin from the kidney on circulating angiotensinogen (12,182). Angiotensin II also increases water intake and stimulates aldosterone secretion (182).

A.2.5.2 Bradykinins. Three related vasodilator peptides called kinins (short-chain peptides, 9 or 10 amino acids) are found in the body (172). The nonapeptide bradykinin is formed in the plasma and the decapeptide lysyl-bradykinin is formed in tissues. Methionyllslylbradykinin has been found in urine. There are high and low molecular weight kininogens in plasma and tissues, but most of the kinins formed in plasma are from high molecular weight substrates, whereas it appears that most of the lysylbradykinin formed in tissue comes from low molecular weight precursors. The kinins are formed by the action of proteolytic enzymes called kallikreins (134).

The actions of kinins resemble those of histamine (134,172). They cause contraction of visceral smooth muscle, but they relax vascular smooth muscle, lowering blood pressure. They also increase capillary permeability. They also attract leukocytes and cause pain upon injection under the skin. They are potent vasodilators and appear to be formed during active secretion in sweat glands, salivary glands and the exocrine portion of the pancreas. They are probably involved in the production of local vasodilation in other active tissues as well. Kinin release is inhibited by adrenal glucocorticoids. A kinin-like peptide has been implicated as a possible cause of migraine (186).

The first and only bradykinin isolated from American amphibians is phyllokinin, which is nothing but bradykinin prolonged at its C-terminus by the dipeptide Ile-Tyr(HSO₃) (39). Phyllokinin is typical for phyllomedusid frogs. Very large amounts of one or more bradykinin-like peptides, certainly different from authentic bradykinin, occur in the skin of *Ascaphus truei*. Expressed in terms of bradykinin, contents varied according to the test system used for the assay.

A.2.5.3 Delta Sleep-Inducing Peptide. The CSF of sleeping animals contains one or more substances that bring about sleep

when infused into the CSF of assay animals (96). One such peptide, named delta sleep-inducing peptide, is a nonapeptide and initially created much scientific interest, but proof that it is released during sleep or is contained in neuronal pathways has not been forthcoming (96).

A.3 ENDOTHELINS/SARAFOTOXINS.

Endothelin (ET) refers to a family of acidic, 21-amino acid peptides found in at least four distinct isoforms: ET-1, ET-2, ET-3, and endothelin β (also called vasoactive intestinal contractor) (119,138,181). ET isopeptides share sequence homology and a common structural design (138); all possess two intrachain disulfide bridges between the cysteine residues 1 and 15, and 3 and 11 (34). Endothelin-2 shows the greatest homology with ET-1 and differs at only 2 positions (119). ET-3 has 6 substitutions relative to ET-1, 4 within the smaller intramolecular loop and two substitutions adjacent to bridge forming cysteinyl residues (119).

Both in structure and bioactivity, ET peptides are closely related to sarafotoxins S6 (a, b, c and d), peptide toxins isolated from the venom of an Egyptian asp (138). Not only is sarafotoxin S6b a potent vasoconstrictor but like the endothelins it also contains two disulfide bridges and a hydrophobic carboxyl terminus with conservation of the terminal tryptophan residue (119).

A.3.1. Endothelins.

Endothelin is equipotent as a vasoconstrictor with angiotensin II and vasopressin and 10-100 times more potent than norepinephrine (26). Endothelins cause the release of a variety of other vasoactive peptides, including endothelial-derived relaxing factor (nitric oxide or a closely related molecule derived from L-Arginine), prostacyclin (a prostanoid vasodilator), and atrial natriuretic peptide (ANP, a natriuretic and vasoactive peptide from cardiocytes) (16,21,38,74,115,119). ET-1 has been reported to increase and decrease renin release and to inhibit the action of vasoactive intestinal polypeptide (16,95,119). In addition, ET-3 elicits a concentration-dependent inhibition of prolactin secretion and stimulates the release of luteinizing hormone, follicle stimulating hormone and thyroid stimulating hormone and thus appears to have an important role as a neuroendocrine modulator (57). ET-3 modulates the release of other peptide hormones, including arginine-vasopressin and substance P (42,181). Increased levels of endothelins have been associated with a variety of disease states (119).

The ET isopeptides show different biological activities (34,64,71,79,86,117). ET-2 is the most potent of the three with regard to the long-lasting pressor and maximum vasoconstrictive

responses. ET-1 is the next potent (79). ET-3 is the least potent. However, the effects of the ET isopeptides on blood vessels are basically similar but quantitatively different (79). For contracting activity the ranking is ET-1=ET-2>ET-3. The diverse effects of ET isoforms are due to multiple subtypes of endothelin receptors (100,112,119,141,159). ET receptors are widely distributed not only in the vascular system but also in such tissues as lungs, kidneys, adrenal glands and neurons. ET receptors on these tissues probably vary from tissue to tissue in their subtypes.

Endothelin-1 binding sites have been identified in coronary arteries, in the walls of small vessels, in the adventitia and associated with nerve trunks (119,147,148,154). Thus ET-1 has receptors at both vascular and nonvascular sites. The presence of sites are reported in cardiac tissue (nerves>atria>ventricles>coronary vessels), renal tissue (glomeruli>papillae), adrenal glands (zona glomerulosa>medulla), central nervous system, lungs and GI tract (119). The receptors have different binding capacities for the different ETs as well as sarafotoxin S6b and VIC (119,159).

There is extensive pharmacological and physiological evidence that ET-1 influences airway caliber (21,32,44,51,70,71,73,80,86,112,113,118,142,156). In mammals, ET receptors occur on airway smooth muscle, local storage and release of the peptide have been demonstrated, and inhalation of ET-1 induces bronchoconstriction independent of cyclooxygenase products (51,71).

Endothelin isopeptides, particularly ET-1, represent the most potent constrictors of guinea pig tracheal and smooth muscle (80,112). Human bronchial smooth muscle cells possess a single class of specific binding sites for ET-1 that belongs to the G protein-coupled receptor superfamily (80). The other two isopeptides of endothelin can also induce bronchoconstriction in humans, although they appear to be less potent than ET-1 in this respect (112). When administered by iv, ET-1 is one of the most potent spasmogens for the trachea and evokes a dose dependent bronchoconstriction accompanied by a marked and sustained increase in mean arterial blood pressure (112). When administered by aerosol, ET-1 also induces a potent bronchoconstriction, whereas no significant change in Mean Blood Pressure is noted (112). Injection of ET-1 via the pulmonary artery provokes a significant increase in pulmonary inflation pressure and pulmonary perfusion pressure associated with the release of eicosanoids, especially thromboxane A₂ and PGI₂ (112). These bronchopulmonary effects of ET-1 (injected intra-arterially) appear to be mediated via the generation of cyclooxygenase metabolites. ET-1 has been shown to constrict the

pulmonary vascular bed independent of the formation of any cyclooxygenase products (71).

Whether the polypeptide is administered by aerosol or it is applied topically, bronchiolar and tracheal smooth muscle respond by developing a sustained contraction (70,112). Studies in rabbits have shown that ET-1 AND ET-3 constrict the pulmonary vascular bed and dilate the systemic vascular bed independent of formation of cyclooxygenase products (71). The systemic hypotensive response to ET-1 is not subject to the first-pass pulmonary metabolism, and the systemic dilator response to ET-1 and ET-3 does not depend on activation of muscarinic, β_2 -adrenergic and PAF receptors (71). In addition, the pulmonary vasoconstrictor response to ET-1 is greater than to ET-3 and both pressor responses are dependent on an extracellular source of calcium (44,71).

Despite the long-lasting responses produced by the iv administration of endothelins to animals, the plasma half-times of the ETs are short (60% removed from the circulation within the first minute, however, the remainder has a half-life of around 45 minutes) (181). No metabolites are located in the plasma and the peptides appear to be taken up by the lungs, kidneys and liver (181). The characteristically long-lasting pressor responses evoked by the endothelins in whole animals is thus not due to their persistence in the plasma (181). It may be due to the slow dissociation from the receptors.

A.3.2 Sarafotoxins.

The four known sarafotoxins have been reported to be highly lethal, causing cardiac arrest within minutes of iv administration (84). S6a and S6b have equal potent smooth contractile activity whereas S6c and S6d have less activity (84). S6a and S6c have been shown to increase lobar arterial pressure in a dose related manner and elicit biphasic changes in systemic arterial pressure when injected in doses of 0.03-0.3 nM (84). The mechanism by which the sarafotoxins cause death include coronary constriction accompanied by ST-segment elevation, a slow positive inotropic effect and atrio-ventricular block (181).

A.3.3 Vasoactive Intestinal Contractor.

More recently 2 endothelin-related genes have been identified in the mouse genome, one of which corresponded to the preproendothelin-1 gene and the second, a novel sequence, codes for a 21 amino acid peptide containing 2 disulfide bridges in identical positions to those in the endothelins (16,56). This novel peptide differs from ET-1 by 3 residues located within the intramolecular loops; the gene for this peptide is expressed only

in the intestine; and the peptide has been designated vasoactive intestinal contractor (VIC) (16).

A.4 CYTOKINES.

Cytokines are polypeptides synthesized by many cells which act on a variety of tissues by changing gene expression and cellular metabolism and help sustain, amplify and regulate the cellular immune and inflammatory response to local infection (28,33,35,111, 114,121,123,164,168,171,177). Cytokines are capable of affecting the function of virtually every cell, tissue, and organ system (171). They have endocrine, paracrine, and autocrine roles in the inflammatory response and mediate changes that resemble aspects of sepsis and injury (171). Many cytokines have similar, or at least overlapping, activities. But even molecules that have a multiplicity of activities in common may have different consequences to the host (171). For example, interleukin-1 appears to participate in protecting the organism from an array of insults, whereas tumor necrosis factor, not only may be lethal to tumor cells, but, in some instances, to the host itself (171).

The pro-inflammatory cytokines are a particular group of cytokines with molecular weights between 8,000 - 25,000 Da which appear to be synthesized primarily in association with disease states or during host perturbation (171). These polypeptides are very potent molecules which, at picomolar or even femtomolar concentrations, trigger a variety of responses in cells (171). The cytokines are synthesized in one cell and act on adjacent or distant cells. Cytokines appear to fall into two main groups: cytokines that act primarily as growth factors for a variety of cells and cytokines that possess pro-inflammatory properties (171). Examples of cytokines which act as growth factors are IL-2 and IL-4. TNF and IL-1 are two of the most thoroughly studied pro-inflammatory cytokines (171). Fibroblast growth factor, transforming growth factor beta, interferons and IL-6 also possess biological activities which are associated with inflammatory states.

IL-1, TNF, IFN α and IL-6 are endogenous pyrogens and produce fever in animals and humans (171). The pro-inflammatory cytokines, particularly IL-1 and TNF, stimulate gene expression for cyclooxygenase and phospholipase A₂ and as a result of these effects, prostaglandin and leukotriene synthesis remain elevated in tissues for several hours (171). In addition, IL-1 and TNF stimulate the expression of each other's gene as well as those of other cytokines, including IFNs, IL-6 and IL-8.

Despite similarities in the biological responses to the various pro-inflammatory cytokines, each has its own receptor molecules (13,30,50,168,171). The receptors for the pro-

inflammatory cytokines such as IL-1, TNF and IL-6 appear to be multiple in that there are separate genes coding for distinct molecules. Therapeutic uses for these cytokines are being tested in humans.

A.4.1 Interleukins.

Interleukin-1, a peptide produced by activated macrophages, affects nearly every tissue and organ system and has many different biological activities, including stimulation of IL-2 release by T cells, stimulation of B-lymphocyte proliferation and maturation, stimulation of proliferation of fibroblasts, and stimulation of prostaglandin and collagenase release by synovial cells (3,4,10,14, 15,17,31,37,124,136,168,171,177). Humans produce two different IL-1 proteins with distinct but partially homologous amino acid sequences (24,168,171,177). Thus the two proteins have been named IL-1 α and 1 β . IL-1 belongs to a group of cytokines with overlapping biologic properties (171). These are tumor necrosis factor and IL-6. IL-1, TNF and IL-6 share the ability to stimulate T and B lymphocytes, augment cell proliferation, and to initiate or suppress gene expression for several proteins (171). Like IL-1 and TNF, IL-6 is an endogenous pyrogen and an inducer of acute phase response (168,171).

IL-1 is the prototype of the pro-inflammatory cytokines in that it induces the expression of a variety of genes and the synthesis of several proteins that, in turn, induce acute and chronic inflammatory changes (171). IL-1 is also the prototype "alarm" cytokine in that it brings about increases in a variety of defense mechanisms, particularly immunologic and hematologic responses (168). IL-1 is both a mediator of disease and a mediator of the host defense (75,171). Over-production leads to debilitation of normal host functions. A single injection into animals of 10 to 100 ng/kg of either IL-1 form results in fever neutrophilia, and increased circulating levels of colony-stimulating factors. The systemic administration of the same dose of IL-1 into humans has produced fever, sleepiness, anorexia, generalized myalgias, arthralgias, headache and some gastrointestinal disturbances. At higher doses, hypotension has been observed.

Low doses (1 -10 ng/kg) of IL-1 in humans cause an increase in the number of circulating neutrophils and platelets as well as increased levels of hemopoietic stem cells (171). However, IL-1 also causes fever, joint pain, and a state of lethargy. At higher doses (100 ng/kg) IL-1 reduces appetite, induces GI disturbances and causes hypotension that can reach dangerous levels when more than 300 ng/kg is given (171).

IL-1 particularly IL-1 β acts in the CNS as a neuromodulator, but does not cross the blood-brain barrier (17,37,124,171). Several centrally mediated biologic actions of IL-1 require the

integrity of prostaglandin pathways. A subpeptide of IL-1 β from 208-240 (amino acid numbers refer to pro-IL-1) possess sleep and pyrogenic properties but lacks T-cell activation (171). Other subpeptide units (5 to 9 amino acids) exhibit other actions of IL-1 but lack the pyrogenic and sleep characteristics.

A.4.2 TNF.

Human tumor necrosis factor/cachectin (TNF) is a 17 kDa polypeptide cytokine composed of 157 amino acids (157,171,177). The biology of TNF represents a spectrum of activities that ranges from inflammation and wound healing to wasting and shock (5,20,39, 43,52,55,72,81,102,105,136,153,157,171). Once released into the circulation or tissues, the biologically active form of TNF is a heterotrimer that mediates a diverse range of inflammatory and metabolic effects on body tissues by interaction via one of two specific membrane receptors (20,39,55,177). TNF's biological activities include fever, stimulation of bone-resorption, stimulation of IL-1 production, differentiation of leukemia cells, stimulation of collagenase and prostaglandin E₂ production, suppression of lipoprotein lipase activity, induction of tissue injury and shock, and many other properties that profoundly affect mammalian cell physiology (157,171,177). The conditions under which exogenous TNF exerts primarily beneficial or deleterious effects are complex and may be dependent on dose, route of administration, and a number of physiological conditions.

The biologic properties of TNF share remarkable similarities to those of IL-1, particularly the non-immunologic effects of IL-1 (171,177). Similar to IL-1, TNF induces fever by its direct ability to stimulate hypothalamic PGE₂ synthesis. On a weight basis TNF is more potent than IL-1 in producing endotoxin shock-like syndrome (171). TNF is a primary mediator in the pathogenesis of infection, injury and inflammation, and in the beneficial processes of host defense and tissue homeostasis (177). Depending on its concentration, duration of cell exposure, and the presence of other mediators in the cellular environment, the net biological effect of this hormone on the host may be beneficial or deleterious: its effects range from the mediation of tissue remodeling, to inflammation, cytotoxicity, cachexia, tissue injury, irreversible shock, and death (157, 171,177).

TNF given intravenously to animals at doses that were similar to the quantity produced endogenously during infection, induced a syndrome of shock and tissue injury that was hemodynamically, hematologically, and pathologically similar to septic shock syndrome (153). Animals succumbing to TNF-induced shock developed hypotension, tachycardia, tachypnea, and a profound metabolic acidosis with fivefold increase in serum

lactate. At necropsy there was evidence of diffuse hemorrhagic necrosis in bowel, acute renal tubular necrosis, pulmonary leukocyte margination, and edema. TNF also triggered the development of a systemic capillary leak causing a large intravenous fluid requirement to maintain cardiac filling pressures and blood pressure (153). These hemodynamic changes persist for up to several days after the administration of a single dose of TNF. The toxicity of TNF is enhanced by IL-1 (153). IL-1 is minimally toxic, even when given at very high doses, but when coadministered with normally nontoxic doses of TNF the synergistic combination of the two cytokines is deadly. Besides IL-1, other cytokines have been studied for their role in modulating the effects of TNF (153). IL-6, PAF and IFN γ may enhance TNF's injurious effects, but transforming growth factor beta attenuates them. The acute overproduction of TNF and the development of septic shock is an uncommon event in mammalian life (171). Usually the production and actions of TNF are confined to its paracrine (beneficial) effects in tissues.

Clinical trials of inhibiting TNF in patients with septic shock syndrome are underway (180,177). Pharmaceuticals are being investigated that may modulate its activities at the cellular level.

A.4.3 Interferons.

IFNs, initially characterized for their ability to "interfere" with viral replication, are functionally related proteins and glycoproteins which are classified into three different types on the basis of their physicochemical and biological properties: leukocyte or interferon-alpha (IFN- α), which is a polypeptide of 165 to 166 aa ~19 kDa, fibroblast or interferon -beta (IFN- β) ~ 20 kDa, and immune or interferon-gamma (IFN- γ), which contains 133 to 136 aa ~15200 Da and is produced by lymphocytes (168,177).

In human, when high doses of IFNs are administered, several adverse effects are induced in a variety of systems (168). Subjects receiving IFN therapy show several alterations in behavior and sleep patterns. These neurotoxic effects include fever, fatigue, anorexia, dizziness, impaired cognition, mood alterations and CNS depression.

A.5 OTHER GASTROINTESTINAL HORMONES.

Many different hormonally active polypeptides have been isolated from the GI mucosa. When large doses of these hormones are given, their actions overlap (183). However, their physiologic effects appear to be relatively discrete. On the basis of structural similarity and, to a degree, similarity of function, many of the hormones fall into one of two families: the gastrin family, the primary members of which are gastrin and

cholecystokinin (CCK); and the secretin family, the primary members of which are secretin, glucagon, glicentin, VIP and gastric inhibitory peptide (GIP).

Gastrin is typical of a number of polypeptide hormones in that it shows both macroheterogeneity and microheterogeneity (172). Macroheterogeneity refers to the occurrence in tissues and body fluids of peptide chains of varying length; microheterogeneity refers to differences in molecular structure due to derivitization of single amino acid residues. Progastrin is processed into fragments of varying size. Three main forms of gastrin (G 34, G 17, G 14) contain 34, 17, and 14 amino acid residues respectively. All have the same C-terminal configuration (172). Another form is the C-terminal tetrapeptide and there is also a large form that is extended at the N-terminal and contains more than 45 amino acid residues (172). One form of derivitization is sulfation of the tyrosine that is the sixth amino acid residue from the C-terminal. Another derivitization is the amidation of the C-terminal phenylalanine (172).

APPENDIX B

ABBREVIATIONS

aa - amino acid
AA - arachidonic acid
ACTH - adrenocorticotropin hormone
ADP - adenosine di-phosphate
ala - alanine (amino acid)
Ang - angiotensin
ANP - atrial natriuretic peptide
arg - arginine (amino acid)
ATP - adenosine tri-phosphate
AVP - arginine-vasopressin
BBB - blood-brain barrier
CCK - cholecystokinin
CSF - cerebrospinal fluid
CNS - central nervous system
cys - cysteine
Da - dalton
DNA - deoxy-ribonucleic acid
DSIP - delta sleep-inducing peptide
E - endorphin
ELISA - enzyme-linked immunosorbent assay
ET - endothelin
fg - femtogram
g - gram
GC - gas chromatography
GIP - gastric inhibitory peptide
GI - gastrointestinal
gly - glycine (amino acid)
GRP - gastrin-releasing peptide
ic - intracerebral
icv - intracerebro-ventricular
IFN - interferon
IGF - insulin-like growth factor
IL - interleukin
Ile - isoleucine (amino acid)
iv - intravenous
kDa - kilodalton
kg - kilogram
leu - leucine (amino acid)
LT - leukotriene
LTA - leukotriene A
LTB - leukotriene B
LTC - leukotriene C
LTD - leukotriene D
LTE - leukotriene E
LXA - lipoxin A
LXB - lipoxin B
LXC - lipoxin C
LXD - lipoxin D

LXE - lipoxin E
met - methionine (amino acid)
 μ g - microgram
 μ m - micrometer
mRNA - messenger RNA
MS - mass spectrometry
ng - nanogram
NKA - neurokinin A
PAF - platelet activating factor
PG - prostaglandin
PGA - prostaglandin A
PGB - prostaglandin B
PGC - prostaglandin C
PGD - prostaglandin D
PGE - prostaglandin E
PGF - prostaglandin F
PGI - prostaglandin I (prostacyclin)
phe - phenylalanine (amino acid)
PP - pancreatic polypeptide
RIA - radio-immunoassay
RNA - ribonucleic acid
sc - subcutaneously
SS - somatostatin
TNF - tumor necrosis factor
TXA - thromboxane A
TXB - thromboxane B
tyr - tyrosine (amino acid)
VIC - vasoactive intestinal contractor
VIP - vasoactive intestinal peptide

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August 1, 2001

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The Defense Threat Reduction Agency Security Office reviewed the following documents in accordance with the Deputy Secretary of Defense Memorandum entitled, "Department of Defense Initiatives on Persian Gulf War Veterans' Illnesses" dated 22 March 1995, and determined that the documents were unclassified and cleared for public release:

DNA-TR-93-84, AD-B244408, Acoustic Resonance Spectroscopy in CW Verification Tooele Field Trial (August 1992).
DNA-TR-93-129-V1, AD-B192045, Global Proliferation – Dynamics, Acquisition Strategies and Responses, Volume 1 – Overview.
DNA-TR-93-129-V2, AD-B192046, Global Proliferation – Dynamics, Acquisition Strategies and Responses, Volume 2 – Nuclear Proliferation.
DNA-TR-91-216, AD-B163637, Harmonizing the Chemical Weapons Convention with the United States Constitution.
DNA-TR-92-180, AD-B175230, Evaluation of the Concept of a List for the BWC.
DNA-TR-92-61, AD-B167663, Basic State Party Functions and Skills Under CWC.
DNA-TR-92-66, AD-B167357, Domestic Reporting Requirements for Chemical Industry.
DNA-TR-91-213, AD-B163260, Analysis of the Interactions Between Treaties.
DNA-TR-93-70, AD-B177262, Chemical Weapons Convention Inspections of Private Facilities Application of United States Environmental and Safety Laws.
DNA-TR-92-182, AD-B173450, Commercial Products from Demilitarization Operations.
DNA-TR-91-217-V3, AD-B169350, Chemical Weapons Process Parameters, Volume 3 – Users' Guide.
DNA-TR-92-116-SUP, AD-B175292, Technical Ramifications of Inclusion of Toxins in the Chemical Weapons Convention (CWC), Supplement.
DNA-TR-92-128, AD-B175452, Task 1 Report Target Vapor Identification and Database Development.
DNA-TR-92-196, AD-B174940, Task 2 Report Algorithm Development and Performance Analysis.
DNA-TR-93-68, AD-B178109, CW Detection Instrument R&D Design Evaluation.

Enclosed is a copy of the referenced memorandum. If you have any questions, please call me at 703-325-1034.

Arndith Jarrett

ARDITH JARRETT
Chief, Technical Resource Center